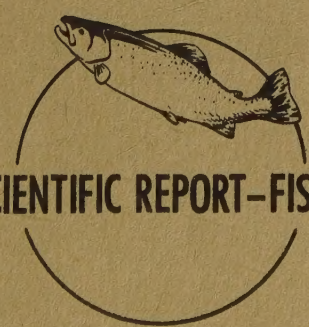
REACTION OF TUNAS TO STIMULI, 1952-53



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United States Department of the Interior, Douglas McKay, Secretary
Fish and Wildlife Service, John L. Farley, Director

REACTION OF TUNA TO STIMULI, 1952-53

Part I: Response of tuna to chemical stimuli, by Albert L. Tester, P. B. van Weel, and John J. Naughton

Part II: Response of tuna to visual and visual-chemical stimuli, by Sidney C. Hsiao and Albert L. Tester

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CONTENTS

Page

Part I: Response of tuna to chemical stimuli..... 1

Fishing..... 2

Establishment of tunas in captivity..... 3

Concrete tank..... 3

Pond No. 5.....4

Feeding.....5

Methods and procedures in testing.....7

Concrete tank.....7

Pond No. 5..... 11

The response.....13

Problems in testing.....16

Weather 16

Power failure.....17

Erratic behavior of the fish.....17

Measurement of response 18

Variation in response..... 24

Source and preparation of test substances.....27

Materials 27

Preparation..... 28

Response to simple extracts of tuna and other fish.....30

Flesh extracts of tuna.....30

Flesh extracts of other fish.....32

Page

Extracts of tissues other than flesh..... 32

Cannery by-products: preservation..... 33

Response to chemical compounds and miscellaneous materials..... 34

Attempts at isolation and identification of the attractant(s)..... 37

Summary and discussion..... 57

General..... 57

Nature of the attractive substance..... 58

Preservation of the attractant..... 59

Possibility of conditioning of the fish..... 59

Literature cited..... 62

Part II: Response of tuna to visual and visual-chemical stimuli..... 63

Methods..... 63

Response under control and experimental conditions..... 67

Pattern of response..... 67

School entrances..... 70

Percentage of Time in Area..... 70

Fish-seconds in area..... 71

Speed of swimming..... 72

Feeding activity..... 73

Response to lures of different colors..... 74

Summary..... 76

Appendix: A summary of experiments on chemical stimulation conducted in tank and pond..... 77

ILLUSTRATIONS

Figure — Page

1. Temperature (°C.), chlorinity (p.p.m.), and mortality (number of fish dying) in Pond No. 5 over the period from September 1, 1952 to June 30, 1953 6

2. Diagram of the concrete tank showing the arrangement for introducing the test materials, the attraction area, and the observation booth 8

3. Number of passes and time (seconds) out of successive 2-minute periods for three tank experiments - Nos. 57, 58, and 59 10

4. Diagram of Pond No. 5 showing the arrangement for introducing test materials, the attraction area, and the observation tower 12

5. Number of passes and time (seconds) out of successive 3-minute periods in six pond experiments - Nos. 136 to 141, inclusive 14

6. Number of passes and time (seconds) out of successive 3-minute periods in seven pond experiments - Nos. 94 to 100, inclusive 20

7. Time (seconds) out of successive 3-minute periods in pond experiment No. 112, in which a gradient of stock extract of skipjack flesh was established for 48 minutes 31

8. Fractionation of stock extract of skipjack flesh: procedure and results in experiments Nos. 83 to 86, and 88 40

9. Fractionation of an alcohol extract of skipjack flesh: procedure and results in experiments Nos. 178, 179, 181, and 182 44

10. Fractionation of an alcohol extract of skipjack flesh: procedure and results in experiments Nos. 200 to 202, and 204 to 206 45

11. Fractionation of alcohol extracts of skipjack flesh: procedure and results in experiments Nos. 215, 216, 218, and 223 to 225 47

Figure Page

12. Fractionation of an alcohol extract of bigeye flesh: procedure and results in experiments Nos. 261, 262, 266 to 269, 272, and 277 49

13. Fractionation of a first alcohol extract of bigeye flesh: procedure and results in experiments Nos. 273 to 276, and 278 51

14. Fractionation of a second alcohol extract of bigeye flesh: procedure and results in experiments Nos. 280 to 283 52

15. Fractionation of a third alcohol extract of bigeye flesh: procedure and results in experiments Nos. 285 to 287 54

16. Fractionation of a second alcohol extract of bigeye flesh: procedure and results in experiments Nos. 298 to 303 56

17. Diagram of mechanical device for lowering lures into water: AB - crosspiece; LL' - lures; U - supporting post; F - fulcrum; C - notch; P - plate to cover notch; W - weight; C - cord 64

18. Diagram of transcribing-recording device: A - lever operated by toggle switch (E) for recording "passes"; B - lever operated by dial (D) for recording number of fish in area; C - timer; F - kymograph 66

19. Typical kymograms: A - under control conditions; B - when stimulated by lures; C - when stimulated by lures plus extract 68

Frontispiece--Tuna Study Pond at Coconut Island, Oahu

PART I

RESPONSE OF TUNA TO CHEMICAL STIMULI

BY

Albert L. Tester, P. B. van Weel, and
John J. Naughton
University of Hawaii

In 1951, exploratory studies of the response of tuna and other fish to chemical, visual, auditory, and electrical stimuli were conducted by P. B. van Weel, S. C. Hsiao, I. Miyake, and A. L. Tester (Special Scientific Report: Fisheries No. 91) of the faculty of the University of Hawaii under contract (No. I6fw-13331) with the U. S. Department of the Interior, Fish and Wildlife Service, Pacific Oceanic Fishery Investigations. It was hoped that these studies not only would contribute to an understanding of tuna behavior but also would provide an indication of ways of attracting tuna to within the reach of a fishing vessel at sea.

Of the various 1951 studies, the most promising was in the field of chemoreception. Van Weel (1952) found that little tunny (Euthynnus yaito) and yellowfin (Neothunnus macropterus) held in captivity responded to clear, almost colorless extracts of tuna flesh.

A new 1-year contract (No. I6fw-18564) for a further study of the reactions of tuna to chemical stimuli became effective June 1, 1952. Efforts were to be made to duplicate and extend van Weel's observations, to identify chemically the attractive substance(s), and to make observations on the reaction of the fish to combined chemical and visual stimuli.

This report deals with the 1952-53 results in the field of chemoreception and includes a brief account of the success attained in fishing and establishing the tunas in captivity.

Note: Contribution No. 47 of the Hawaii Marine Laboratory, University of Hawaii.

The tunas used in the study were caught and transported to Coconut Island by Mr. Lester Zukeran, skipper of the University of Hawaii research vessel Salpa. He was ably assisted by Mr. Stanley Kitagawa and Mr. Royden Ikeda, students at the University. In the ponds, the tunas were fed and otherwise cared for by Mr. Charles Nakamoto, assistant to the Hawaii Marine Laboratory, who also took water temperatures and samples of water for chlorinity determination.

At times, Mr. Austin Pritchard and Miss Shirley Trefz, graduate students in marine zoology, assisted in pond testing. Mr. Carl Swanholm, student in chemistry, assisted in preparing and analyzing test materials.

Samples of chemicals were provided gratis by E. I. DuPont de Nemours and Company through Mr. Raoul Pantaleoni, by Van Ameringen-Haebler, Inc., through Dr. Ernst T. Theimer, by P. R. Dreyer, Inc., through Mr. George H. Zirkel, and by Sindar Corporation through Mr. R. E. Horsey.

Mr. Fritz Jermann of Hawaiian Tuna Packers, Ltd., spent many hours preparing materials for testing and assisted in many other ways. Certain materials were also forwarded by Mr. Pete Sunderland of B. C. Packers, Ltd.

To all of these individuals, to the companies they represented, and to others who assisted in various ways we extend our sincere thanks. We are also indebted to Mr. O. E. Sette, Director, and other members of the staff of the Pacific Oceanic Fishery Investigations, U. S. Fish and Wildlife Service, for advice, assistance, and encouragement.

FISHING

As in the previous year (Tester 1952), tuna were caught by trolling with the 46-foot Salpa, using two lines suspended from each of two poles fastened to the mast amidships, and sometimes using one or two additional lines fastened to the stern. The ship operated three mornings a week off Kaneohe Bay during the period June 6 to August 31, 1952. As shown in table 1, the catch consisted of 63 skipjack (Katsuwonus pelamis), 66 kawakawa or little tunny (Euthynnus affinis), 1/ 33 yellowfin (Neothunnus macropterus), 6 dolphin (Coryphaenus hippurus), and 2 wahoo (Acanthocybium solandri). The catch per hour (all species) was low in June (1.70) but higher in

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1/ Euthynnus yaito of previous reports (Tester 1952; van Weel 1952).

July (2.36) and August (2.40). The catch per hour for the three months (2.17) was less than that for the same three months in 1951 (3.20).

For tunny and yellowfin, the two species of tuna which we have established in captivity, the catch per hour was higher in 1952 (0.84 and 0.42) than in 1951 (0.40 and 0.31) for a comparable period. For skipjack, however, the catch per hour in 1952 (0.81) was considerably less than that in 1951 (1.48).

Fishing was not continued after August 1, 1952, (except for incidental trips) as the tank and pond were stocked with sufficient fish for our purposes.

Table 1.--The number of fish caught, fishing hours, and catch per hour for the 1952 fishing operations of the Salpa, with comparable data for 1951

Month	Number of fish caught							Fishing hours	Catch per hour
	Skipjack	Frigate Mackerel	Tunny	Yellowfin	Dolphin	Wahoo	Total		
June	21	--	8	6	5	--	40	23-1/2	1.70
July	29	--	29	19	--	2	79	33-1/2	2.36
August	13	--	29	8	1	--	51	21-1/4	2.40
Total, 1952	63	--	66	33	6	2	170	78-1/4	2.17
Total, '51 (June to August)	100	17	27	21	52	--	217	67-3/4	3.20

ESTABLISHMENT OF TUNAS IN CAPTIVITY

Profiting by last year's experience, attempts were made to establish only yellowfin and tunny in the concrete tank and in Pond No. 5, which were used successfully in 1951 (Tester 1952). The tank and pond are located on Coconut Island.

Concrete Tank

The concrete tank, which measures 35 x 11 x 4 feet and is supplied with running seawater by pumps, was modified slightly by rounding the corners with sheets of Masonite and painting the walls white. Despite these improvements only 2 out of 11 yellowfin

and 2 out of 13 tunny were established, the former but a short period of time.

Nine yellowfin (3 to 6 pounds) died within 1 or 2 days after introduction to the tank, without starting to feed. One, introduced on June 30 (4 pounds), started feeding on July 4 but was caught and stolen by poachers during the same evening. Another, introduced on July 14 (5-1/2 pounds), started feeding on July 22 but stopped on July 26. In obvious difficulty, it was removed from the tank on July 30 and was found to be blind, with bubbles in the eyeballs.

Eleven tunny (1 to 4 pounds) died within a few days after introduction to the tank. Of these, two started feeding but after 12 days became blind; one died and the other, in obvious difficulty, was removed from the tank. Two tunny, introduced respectively on June 16 and August 15, 1952, became established and lived throughout the winter. They were still alive on June 30, 1953. Both weighed about 1 pound when established and grew to about 4 pounds during the year 2/.

Pond No. 5

This pond, described in detail by Tester (1952), is about 360 feet long and 75 feet wide. The south side is shallow over its entire length, averaging about 3 feet in depth at high water. The north side includes a deep trough, which averages about 10 feet in depth. A slow circulation is effected by tidal action through screened gates at both ends. Both yellowfin and tunny were established in this pond in the summer of 1951 but died during the fall, following wet, stormy weather.

Between July 23 and August 27, 1952, 13 yellowfin (5 to 10 pounds) were placed in this pond. Of these, five started feeding and became established. Four of the 5 disappeared from the pond during the first 2 weeks in September--it is suspected that they were caught by poachers, although they may have died "naturally." In any

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2/ One of these jumped from the tank and died on July 19, 1953. It was partially eaten (by rats or cats?) when discovered, and was not weighed. The other started ramming the walls of the tank and died on July 22, 1953. A film over the eyes indicated partial blindness, which may have contributed to its death when alone in the tank. It weighed 3-3/4 pounds at death.

event, the carcasses were not found. The fifth yellowfin died on September 16, 1952. An additional yellowfin (10 pounds) was placed in the pond on October 28, but it died 5 days later without starting to feed.

Of 15 tunny (1 to 1-1/2 pounds) introduced to Pond No. 5 between July 25 and August 25, 1952, 13 started to feed and became established, despite the fact that several were tagged (the tags later became detached). The remaining 2, which were tagged, died within 2 days. The 13 established tunny were present until October 16, 1952, following which 6 died--3 between October 16 and 20, 2 between October 21 and 27, and 1 between October 30 and 31. The remaining population of 7 fish was maintained until December 5, 1952, when 1 more died. The 6 remaining fish persisted until January 16, 1953. One died on January 16, 2 between January 17 and 20, and 1 between January 22 and 23, 1953. The 2 tunny which remained survived the winter and spring and are still living at the time of writing, more than 1 year after they were introduced. During this period they have increased in weight from about 1 to 4 pounds.

The reason for the mortalities of tunny reported above is uncertain. The temperature and chlorinity in Pond No. 5 are shown in figure 1. The six deaths in October coincided with the beginning of a steep downward trend in temperature and a slight downward trend in chlorinity, both of which were associated with the incidence of rainy weather. However, the four deaths in January cannot be related to changes in temperature and salinity. Moreover, the two remaining fish survived through February and March, when considerable rainy weather was encountered and when the chlorinity in the pond reached a low for the year (15.834 p.p.m. on March 11, 1953).

Although comparable temperature and chlorinity data for the previous year (when all fish died following wet stormy weather) are not available, in general, the winter weather in 1952-53 was much better than in 1951-52, with markedly fewer "kona" storms (south to southeast winds with rain). This may have contributed to the success in maintaining two of the fish throughout the winter. During the one bad storm of the 1952-53 season on October 13 and 14, mortality may have been reduced by tying the Salpa to the dock near the outer gates and flushing fresh water from the pond with the wash of the propeller.

Feeding

In both the tank and pond the tunas were fed during the late afternoon of Tuesday, Thursday, Saturday, and occasionally Sunday, of each week. Their food consisted mostly of tuna flesh

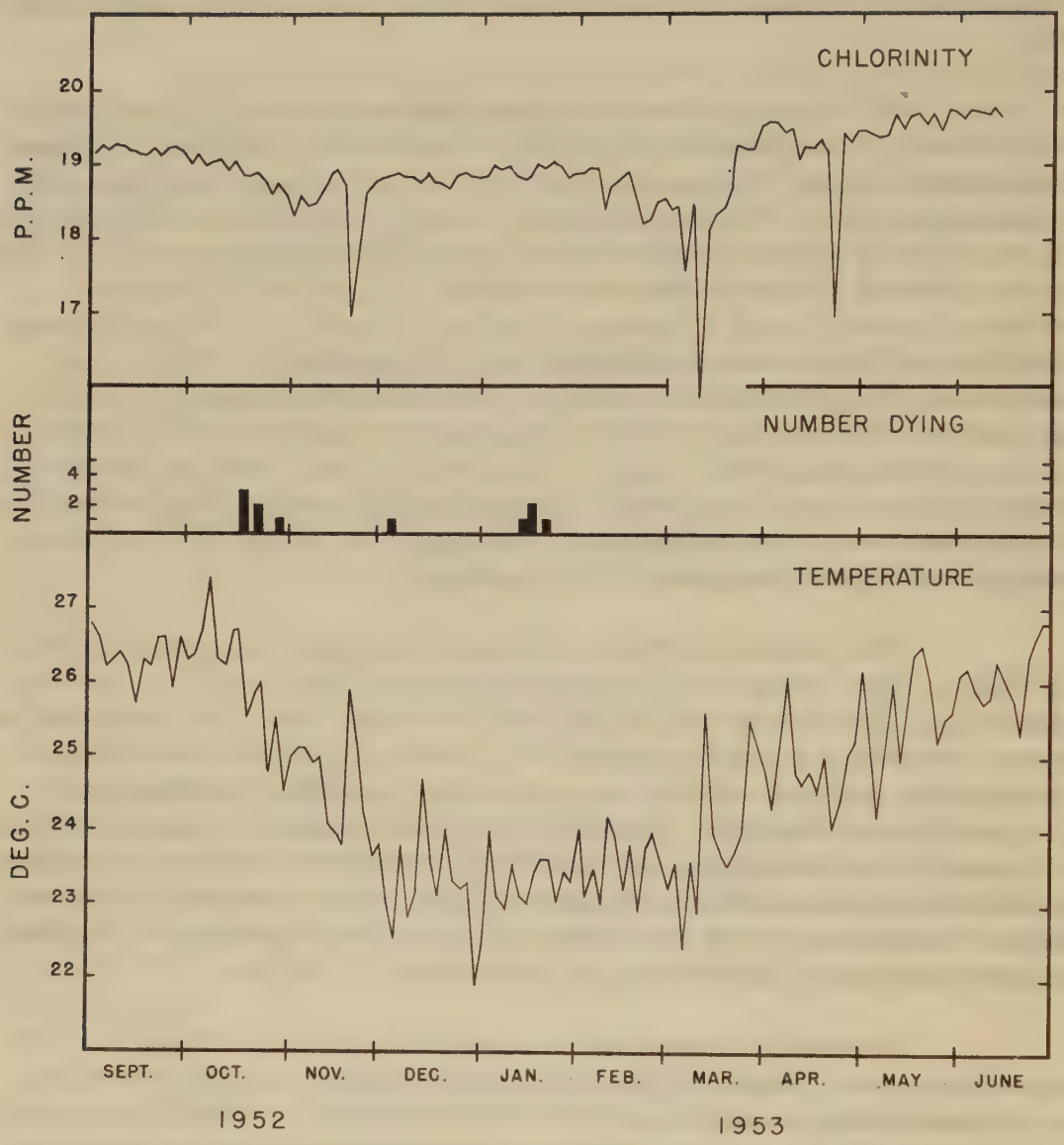


Fig. 1.--Temperature (°C), chlorinity (p.p.m.), and mortality (number of fish dying) in Pond No. 5 over the period from September 1, 1952 to June 30, 1953.

(skipjack, yellowfin, or bigeye) or marlin--whichever was available--plus such other scrap fish as came to hand. In general they were fed at the rate of about one-half pound of food per fish per feeding, which is probably much less than their normal requirement. This schedule was maintained in order that the fish would be in a constant state of hunger on Tuesdays, Thursdays, and Saturdays when tests were usually conducted. Van Weel (1952) showed that this precaution was necessary.

Two departures from this diet are worthy of note. Firstly, the tunny established in the tank on June 16, 1952, was kept on a non-tuna diet, consisting of squid, anchovy, and other fish, until about the middle of July when certain tests had been completed. Secondly, following April 25, 1953, the two tunny in the pond were fed on imported (frozen) squid exclusively.

METHODS AND PROCEDURES IN TESTING

Concrete Tank

Tests were run in the concrete tank 2 or 3 times a week from July 1 to August 20, 1952, and occasionally thereafter throughout the remainder of the summer, the fall, and the winter. At first the methods were similar to those used by van Weel (1952). The observer stood by the railings at the side of the tank near the south end until the fish had become used to his presence and had resumed a steady slow circling. Several successive measurements and counts were made of (a) the time (in seconds) for 10 passes past the observer, with the fish swimming either up or down the length of the tank, and (b) the number out of 10 passes made in the half of the tank adjacent to the observer. Materials were then siphoned into the tank using a rubber hose, and the timing and counting were resumed. Excitement induced when the fish sensed the material was indicated by a decrease in the time of 10 passes (increase in rate of swimming) and attraction was indicated by an increase in the number of passes along the near side of the tank.

Often during attempts to establish control conditions the fish would remain excited for long periods of time by the presence of the observer, whom it could see; during both control and test periods it might also respond to the observer's movements while timing, introducing the materials, or recording the results. This difficulty was largely overcome on July 23, 1952, (following Test 13) by enclosing the upper railed section of the tank with tarpaper and building an observation booth (fig. 2). The three-ply front of the booth overhung the tank at its upper edge to increase the range of vision. It contained

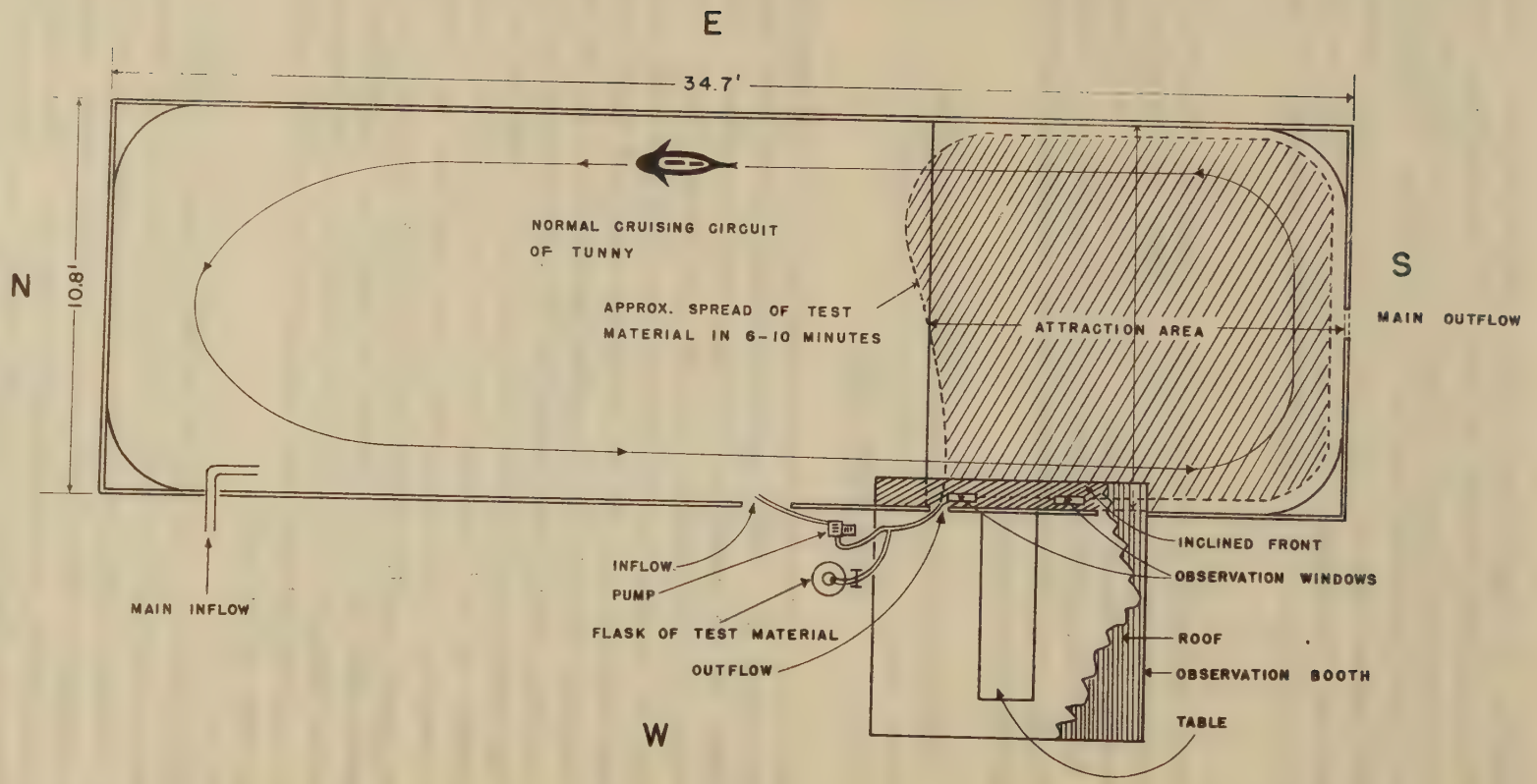


Fig. 2.--Diagram of the concrete tank showing the arrangement for introducing the test materials, the attraction area, and the observation booth.

two rectangular 6 x 10-inch observation windows the size of which could be varied to a narrow horizontal slit by means of sliding doors. Later, in August, the observation windows were equipped with one-way glass. As nearly as could be determined, the fish were generally unaware of the presence of the observer as long as strict silence was maintained. Occasionally, however, before the glass was used it seemed as if the fish were aware of the small part of the observer's face which was visible through the small slit-like aperture.

By means of a 3/8-inch centrifugal pump driven by a 1/30 h.p. near-silent electric motor, water was sucked from the surface of the tank at one side near the center and was injected into the southern or "downstream" one-third in a continuous stream during both control and test periods. When desired, the test material was introduced into this stream from within the booth through a glass T connection in the rubber tubing.

The method of measuring the reaction was modified from that of van Weel (1952). Each test consisted usually of five successive 2-minute control periods, followed by at least five successive 2-minute test periods. For each period, the time spent by the fish in the downstream one-third of the tank, marked off with string, was measured by an electric clock operated by a push switch, and the number of loops (half-circles) made by each fish in this "attraction" area was recorded on a hand counter. By means of fluorescein dye it was found that a liquid quickly spread throughout the downstream one-third of the tank, remained there for about 10 minutes, and then gradually dispersed up the tank along the far wall. If attracted by the material, the fish would be expected to spend a longer period of time in the attraction area as compared with control conditions. If excited by the material the fish would be expected to swim more rapidly and to circle more times in the attraction area than during control conditions. If a fish entered, turned, and left the area, the count would be one loop. If the fish entered, made a complete circle within the area, and then left, the count would be three loops. If two fish performed in this way, either together or independently, the counts would be doubled. Later the method of counting was modified to conform with that in the pond (see below).

The tests in the tank were conducted for the most part on one established tunny during the summer, and on two established tunny thereafter. Unestablished fish did not react to the test substances and were ignored in timing and counting. A typical series of reaction graphs is shown in figure 3.

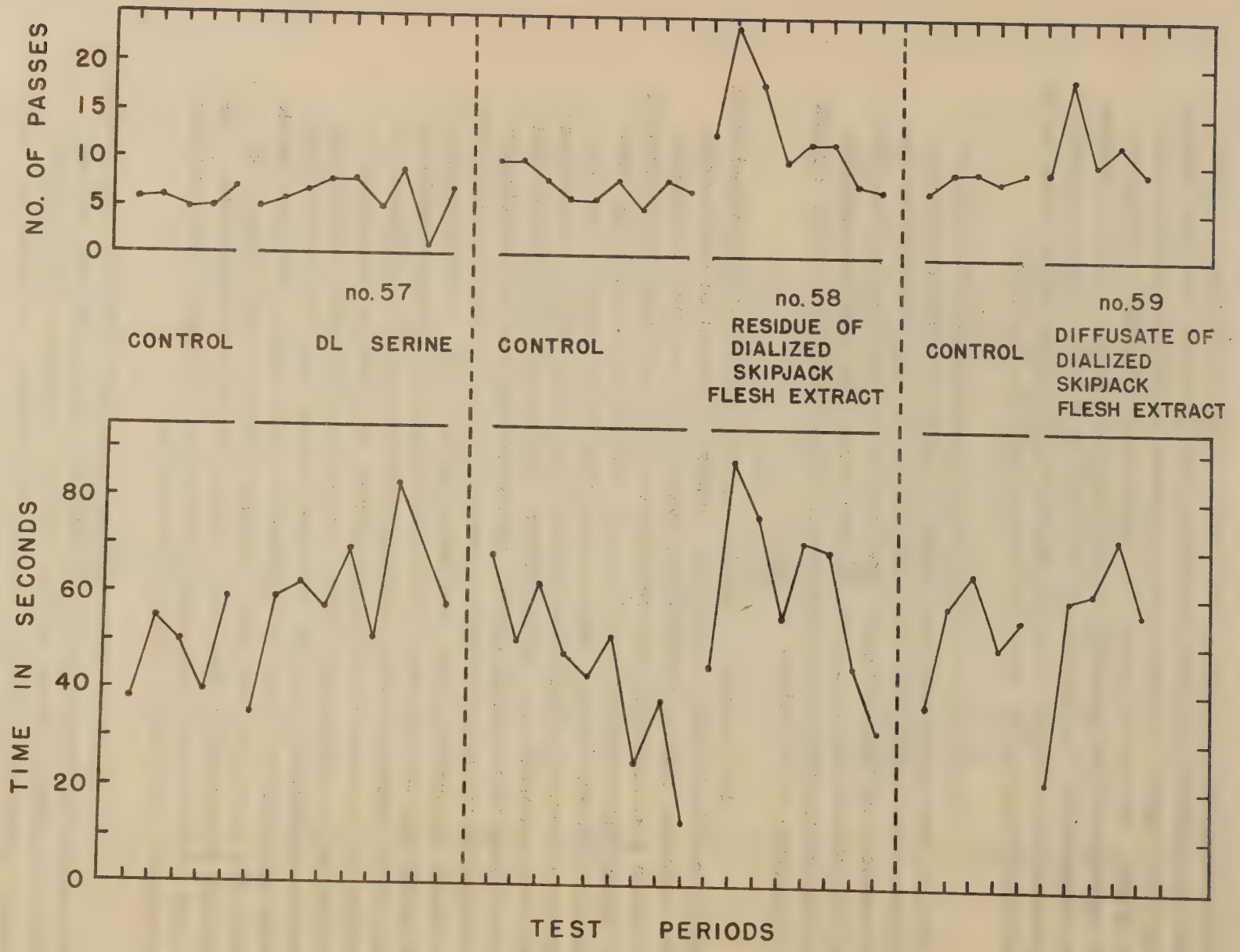


Fig. 3.--Number of passes and time (seconds) out of successive 2-minute periods for three tank experiments--Nos. 57, 58 and 59.

Pond No. 5

When weather and other conditions permitted, tests were run in Pond No. 5 two or three times a week, starting in August 1952 and continuing throughout the fall, winter, and spring. The general arrangement for testing is illustrated in figure 4.

By means of a 2-inch pump driven by a 3-h.p. electric motor, water was sucked from outside the "seaward" gates at the western end of the pond, led through a 2-1/2-inch rubber hose along the concrete gate supports to a point near the center of the deep trough in the pond, and then injected into the pond about 5 feet below the surface (from the high tide mark) through an L-shaped piece of 2-inch galvanized pipe. Test material was introduced into the flow through a funnel, the base of which was attached to a piece of 3/4-inch galvanized pipe by a rubber hose. The 3/4-inch pipe was brazed at an acute angle into a hole in the "down" section of the L-shaped pipe, creating a suction. The funnel was located within a small house erected above the seaward gates, thus effectively screening the operator when waiting to introduce test liquids.

The pump, which was rather noisy and which created a strong visible flow of water for about 20 feet into the pond, was kept running for some time before as well as during both control and test periods to accustom the fish to both the noise and the current. After tracing the path of the current with fluorescein dye, an "attraction" area was marked off with two pieces of cord stretched across the pond, one 39 feet and the other 78 feet from the seaward gates. The dye indicated that liquids introduced into the flow would reach the near edge of this area within about 1 minute, would spread throughout most of the area within about 3 or 4 minutes, and would for the most part remain in the area for an additional 10 minutes before appreciably dispersing downstream across the far cord.

To observe the fish, a wooden tower 20 feet high was built opposite the center of the attraction area on the north side of the pond. A platform and seat on top of the tower was reached by a ladder. As the material was introduced when the fish were starting to return from the far end of the pond, after pouring the material down the funnel the observer was able to move quickly from the small house to the tower, climb the ladder, and sit on the platform before the fish approached the attraction area. It is reasonably certain that he was not noticed by the fish either when moving to the platform or when seated on it. Timing and counting were conducted from the platform. Observation was improved by wearing polaroid sun glasses.

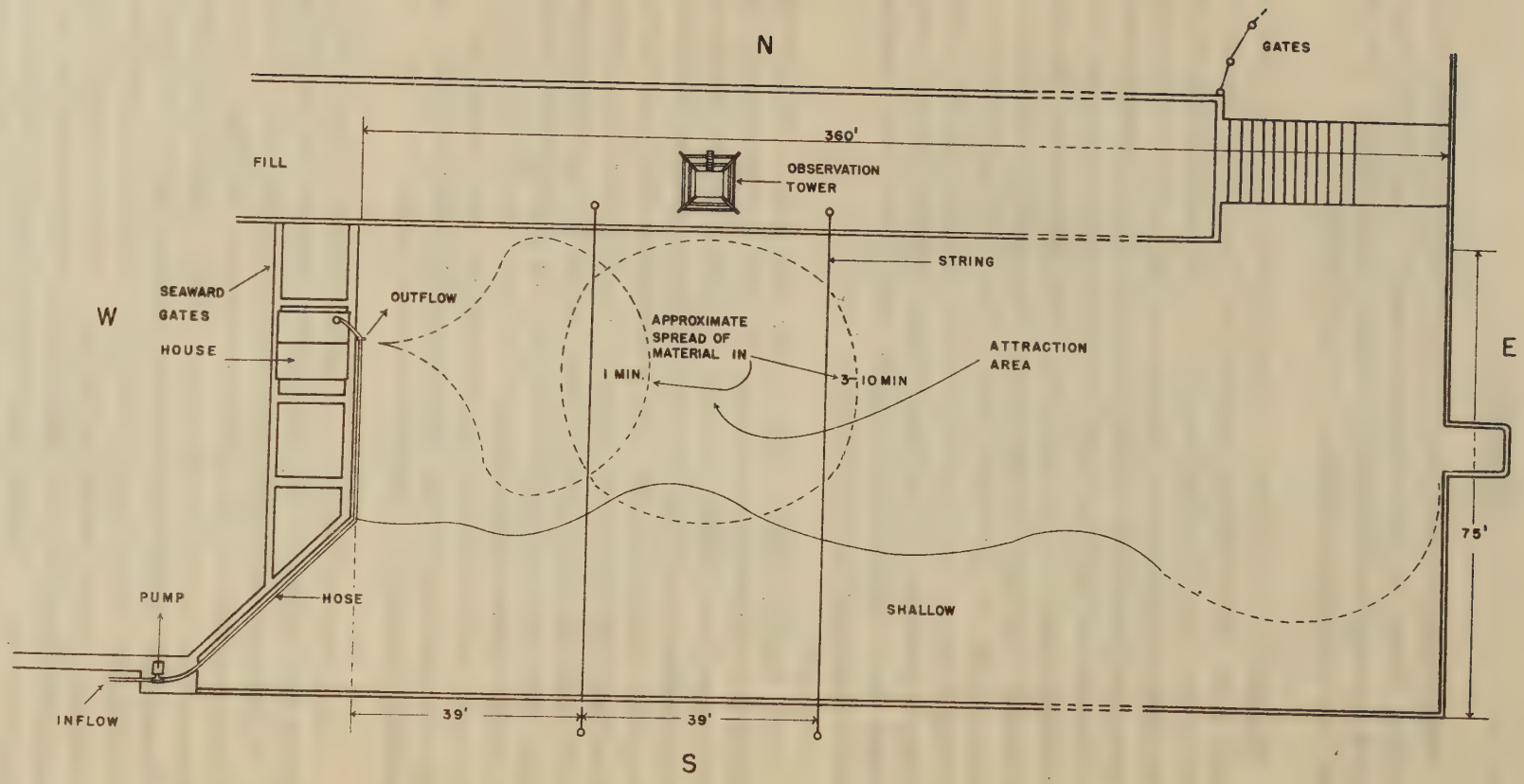


Fig. 4.--Diagram of Pond No. 5 showing the arrangement for introducing test materials, the attraction area, and the observation tower.

Each experiment usually included five 3-minute control periods and five 3-minute test periods. During each period the time spent by the tunny school or schools in the attraction area was measured on an electric clock operated by a push switch. The switch was depressed when the first fish of a school entered the area and was held down until the last fish left. A fish swimming by itself was ignored; often it was a partially-blind or otherwise incapacitated individual which was unable to keep pace with the others. In addition to timing the fish, each "pass" of each fish in either the upstream or downstream direction was recorded on a hand counter. Thus, if three fish swam through the area, the count would be 3. If they entered the area, looped, and left on the same side as they entered, the count would be 6. If they entered the area, described a complete circle within the area and then left on the opposite side to which they entered, the count would be 9. Time spent in the area was accumulated on the clock and the number of passes was accumulated on the counter. Both were recorded at the end of each 3-minute period. When the tunny population was large (6 to 13 fish), two observers were required on the tower, one of whom climbed down and introduced the test material between control and test periods, and returned when the fish were at the far end of the pond to avoid being seen. When the fish were excited during the early part of a reaction it was difficult to record all the movements, even with two observers; in some cases the counts were minimal estimates. As in the case of the tank, the difference between control and test periods in average time spent by the fish in the attraction area was used as a measure of attraction and the difference in count was used as a measure of the excitement induced by the test substance. A typical reaction graph is shown in figure 5.

In both tank and pond, in addition to the quantitative measure of reaction discussed above, the observer also indicated the strength of the response in one of several "observational" categories (- to XXXX). These will be discussed in a later section.

THE RESPONSE

A summary of data on 356 experiments which were conducted in the tank and pond at intervals over the period July 1, 1952, to May 19, 1953, is given in the appendix at the end of this report. The experiments will be referred to by number, e.g. Nos. 168, 170, etc. The results will be discussed in detail in later sections. For the present, attention will be directed at a description of a typical response in the tank and the pond.

When an attractive substance, such as an extract of tuna flesh, was introduced into the attraction area of the tank, the

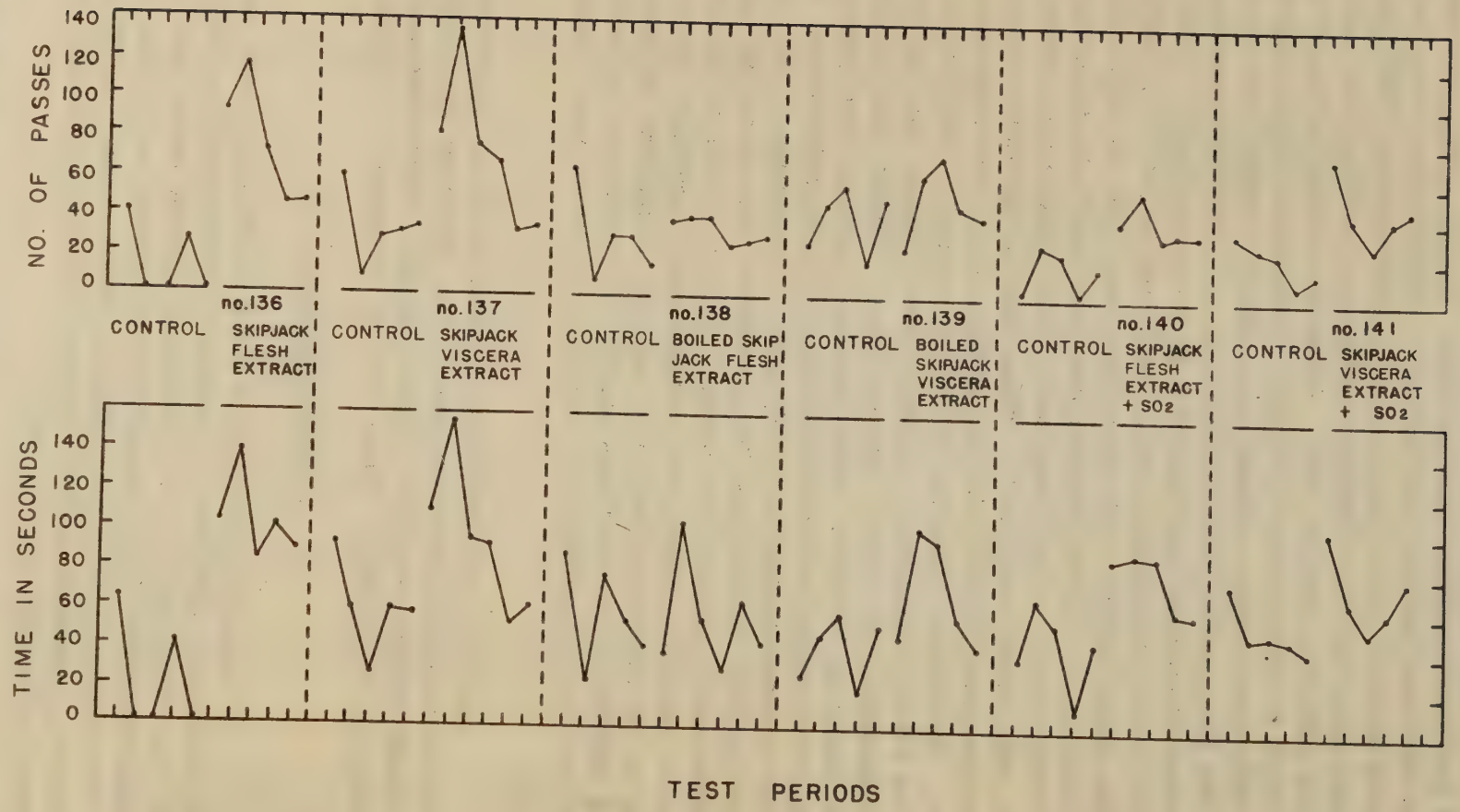


Fig. 5.--Number of passes and time (seconds) out of successive 3-minute periods in six pond experiments--Nos. 136 to 141, inclusive.

material was usually sensed by the tunny in its first pass into the area. Usually the fish would become excited immediately. It would increase its swimming rate, often quickly circling the tank. On returning to the area it would swim rapidly in small circles, splashing and swirling at the surface. At times the fish would flex its dorsal fin and snap with its mouth. Occasionally it would root uneaten food from the bottom and "play" with it (although not eating it as far as could be observed). This excitement would usually be pronounced in the first and second periods (4 minutes from start) but would gradually lessen, until after the fifth period (10 minutes from start) only a more rapid rate of swimming would be noted. If the reaction continued after the fifth period, it would usually take place outside of the attraction area along the far wall, where the material had been carried by the slow, counterclockwise current in the tank.

When an attractive substance was introduced into the pond, a reaction would take place immediately if the tunny happened to be near the point of injection. Usually, however, the material was introduced when the fish were starting to return from the upstream end of the pond. A reaction usually ensued during the first 3-minute period, as soon as the fish entered the attraction area. When several tunny were present, they would immediately depart from a linear, follow-the-leader type of schooling and fan out across the pond, darting into and out of the attraction area at or near the surface, frequently breaking the surface with their backs and creating a "bow wave" as they swam. Often individuals would make short circles in the area, splashing and biting at bubbles, dead leaves, or other objects which happened to be floating on the surface. This activity usually lasted for two periods (6 minutes from start) and often persisted with lessening tempo for five periods (15 minutes from start) or more. When darting into and out of the area, the point at which they turned back seemed to coincide roughly with the dispersion front of the material, the position of which could be inferred from experiments conducted with fluorescein dye. Usually the reaction was over within 15 minutes and normal swimming up and down the pond was resumed. Thus a typical strong reaction was indicated by (a) increased rate of swimming, (b) surfacing, (c) breaking up of usual school formation: a "fanning out" of the school, often followed by independent circling of individuals, (d) return to area after passing through the front of the substance, (e) feeding activity: splashing, lunging, and snapping at objects on the surface.

There was considerable variation in both the pattern and strength of the reaction. Sometimes the fish reacted to the material for but a short time within each period, in the meantime racing the entire length of the pond and quickly returning. When only two tunny

were present the reaction was similar to that described in the foregoing paragraph, but was less violent and less spectacular. Even with a larger number of fish, it could vary in its intensity from the very strong reaction described above to a slight sensing of the material - a mere swerving in the area as the fish passed through. Often only one or two (e.g. a and d above) of the five reaction components were evident. Also, of course, many of the substances which were tested gave no noticeable or measurable response.

PROBLEMS IN TESTING

From the foregoing account of the methods, procedures, and response one might assume that precise, clear-cut conclusions could be drawn from each test. This was not the case. There were several uncontrolled variables which affected the results and which made it difficult, and often impossible, to obtain reasonably consistent and comparable data.

Weather

In the tank, when the sky was overcast the fish were difficult to see and hence to time and count. Several experiments were conducted under unfavorable weather conditions, thus introducing an additional source of error in the quantitative data. Testing during rain squalls was usually avoided, for the rain rippled the surface and made observation very difficult. Occasionally, however, rain occurred while experiments were in progress, thus not only changing the conditions for observation of the fish during test as compared with control periods, but also providing a distraction which might affect the reaction of the fish.

These same difficulties were encountered to even a greater extent in the pond, where the water was deeper, and the fish were difficult to see from the tower when swimming deep. Occasionally, visibility was greatly reduced by southeast "kona" winds, which not only created a turbidity but also, blowing lengthwise down the pond, created ripples on the surface. Often, to avoid wasting a whole day, experiments were conducted under such conditions. Although the resulting data were not strictly comparable with those obtained during fair weather, a positive response during test periods could usually be detected as, when reacting, the fish tended to swim at the surface where they could be seen even under unfavorable weather conditions.

Power Failure

During a 2-week period in November 1952, and occasionally at other times, the power supply to the island and/or to the pumps failed. Rather than waste valuable testing time, pond experiments were conducted without the use of the pump. Test material was introduced by throwing it to the surface from the top of the tower when the fish were distant from the attraction area. It was noted that a material which was usually attractive to the fish was not sensed as soon as when introduced by the pump. Apparently it formed a layer on the surface under which the fish could swim without being aware of its presence. When it was finally sensed, it produced a reaction which was stronger than usual, probably because it was more concentrated than when introduced through the flow from the pump. Again, the data would not be strictly comparable with those obtained when the material was introduced in a normal fashion.

Erratic Behavior of the Fish

In the tank under ideal control conditions the fish would make full circles, keeping close to the walls and swimming at a steady rate. They would thus spend about one-third of their time in the attraction area and each would average about 3 or 4 loops in a 2-minute period. However, ideal control conditions were not often encountered. Usually experiments were run when inspection of the control data indicated fair regularity in movement and thus moderate variance in the data, even though the times and counts might differ considerably from the "ideal." At times, particularly when one tunny was the sole occupant of the tank, it would circle for long periods in either the upstream or downstream end of the tank, giving either zero or maximal times and counts. At times it would circle for several successive minutes at one end and would then repeat the performance at the other end. At other times it would swim back and forth along one wall, entering the attraction area either occasionally or not at all. When behavior patterns such as these were encountered, testing was usually abandoned, with consequent loss of time. Occasionally, in desperation, the material was introduced under such conditions, in the hope that the fish would sense it and show some response even though the data would not be comparable with those obtained with more nearly normal behavior. When two fish were present in the tank, even if one was not yet established, the pattern of movement was more uniform and usually yielded control conditions which did not depart excessively from the ideal.

In the pond, the large black yellowfin were more conspicuous and swam more slowly than the small blue-green tunny.

Although their reactions were noted, they were not measured because of the unsuitable swimming behavior. At times they would circle slowly for hours at one end of the pond; at other times they would circle for long periods at one end, move along one wall to the other end, and repeat the performance; only rarely would they move regularly up and down the length of the pond.

Under ideal control conditions, the tunny would swim up and down the pond in one or two schools at a leisurely but regular pace, occasionally pausing to circle or "play" for a few seconds at the ends. A trip down the pond and back usually required about 3 minutes. When joined by the yellowfin, sometimes the faster tunny and sometimes the slower yellowfin acted as pacemakers, thus varying the swimming speed. At times, either of their own volition or attracted by the yellowfin, the tunny would spend long periods of time, sometimes an hour or more, at one end of the pond. When this occurred, experiments could not be conducted for the test material might be diluted below the threshold for response before the fish entered the attraction area. Several experiments were spoiled when, following uniform control conditions and the introduction of the test substance, the fish failed to return to the area within the required time. Even if they entered the area for the first time during the second or third test period, the quantitative data would not be comparable with those obtained during normal behavior.

Measurement of Response

From the sources of variation discussed so far - weather, variation in testing procedure, and erratic behavior of the fish during either control or test conditions - the difficulty of obtaining consistent quantitative data will be apparent. Moreover, the timing and counting did not measure the more obvious characteristics of a complex positive reaction - surfacing, splashing, and feeding activity. On one occasion the response was recorded on motion picture film, a method which might lead to a better quantitative measure. However, this method was not feasible in regular testing because of the great variation in visibility of the fish (depending on weather, turbidity of the water, height of the tide, and depth of swimming of the fish) and also because of the prohibitive cost of the thousands of feet of film that would be necessary to record the many experiments which were conducted.

A series of pond experiments (Nos. 94 to 100, inclusive) has been chosen to illustrate the nature of the data and the problems of statistical analysis and interpretation under average conditions of observation and behavior of the fish. These seven experiments were undertaken in succession throughout the day of August 26, 1952, four

in the morning and three in the afternoon. The sky was overcast, with a few rain squalls in the morning. Twelve tunny and four yellowfin were present, but only the former were timed and counted. The nature of the test substances is not pertinent to this discussion.

The basic data are shown graphically in figure 6.

It will be noted immediately that there is a high correlation between the times and number of passes for successive test periods ($r = 0.874$; P less than 0.01). Both variates reflect the reactions almost equally well in this particular series. It will also be noted that in all experiments except No. 98, the times and counts during the test periods are generally higher than during the preceding control periods.

Analysis of variance was employed to determine if there were significant differences between the means of control and test periods (equivalent to a "t" test) for each separate experiment. The results are shown in table 2. It may be concluded that there is a statistically significant response to the test substances of experiments Nos. 95, 97, 99, and 100 (P less than 0.05 or 0.01).

In experiment No. 98 the average time and count during the test periods were both slightly less than during the preceding control periods. Obviously, however, this was due to unusual activity during the controls. To overcome this difficulty a more informative method of analyzing the data is to group all control periods for all experiments as T_0, determine the mean square between means for experiment T_0 to T_7, determine the pooled mean square within experiments, and use this last to determine a least significant difference (fiducial interval) of a treatment mean for comparing not only T_1 with T_0, T_2 with T_0, etc., but also for comparing T_1 with T_2, etc. The analysis, for time data only, is as follows:

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Between treatments	7	16,157	2,308	5.0**
Individual periods	62	28,671	462	

** P less than 0.01

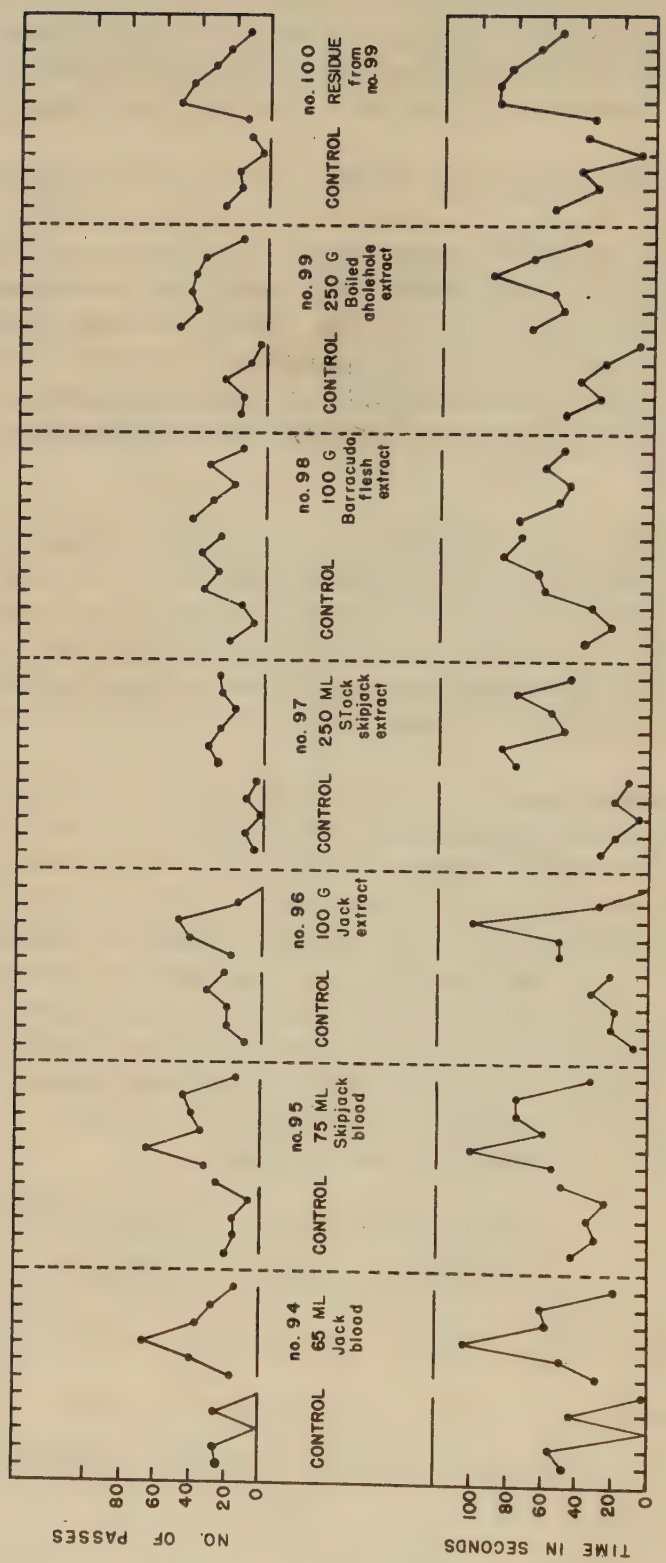


Fig. 6.--Number of passes and time (seconds) out of successive 3-minute periods in seven pond experiments--Nos. 94 to 100, inclusive.

The fiducial interval of a treatment mean may be calculated as follows (Snedecor 1948: p. 221):

$$\pm t_{.05}\sqrt{\frac{s^2}{k_o}}$$

$$= \pm 2.0\sqrt{\frac{462}{7.14}}$$

$$= \pm 16$$

The mean for the controls is 36.2 seconds; those for the experiments are included in table 2. Again, the means for only four experiments, Nos. 95, 97, 99, and 100, as before, differ significantly (by 32 seconds or more) from the mean for the controls. There are no significant differences between the means for any of the seven experiments. This latter conclusion is also evident from a further analysis of the variance of the combined results:

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Treatment vs. control	1	13,138	13,138	28.2**
Treatment means	6	3,019	503	1.1
Individual periods	62	28,671	462	

The above analysis, based on quantitative data, has demonstrated a significant response in four of the seven experiments. Observation, however, showed a response in all seven, namely, obvious sensing of the material combined with one or more of the several components of the reaction, as described previously. Only in No. 98 was there any doubt; even here, however, there was an obvious change in pace and surfacing of the fish during the first and second test periods. The data and their analyses do not always reflect a response which is obvious and "significant" to the observer. Other experiments could be selected in which the discrepancies between the data and the observations are even more pronounced.

The difficulties outlined above were recognized early. Nevertheless timing and counting were continued: they did measure certain phases of the reaction and have been used in some quantitative

Table 2.--Data on a series of seven experiments (Nos. 94 to 100, inclusive) conducted on August 26, 1952, in Pond No. 5.

Experiment No.	Control means		Test means		Difference		F		Sum of time and count differences	Observed response
	Time	Count	Time	Count	Time	Count	Time	Count		
94	30.8	15.8	60.6	37.8	30.6	22.0	3.2	4.6	52.6	X
95	36.6	17.6	73.0	44.0	36.4	26.4	13.1**	15.5**	62.8	XX
96	38.4	20.8	45.4	24.2	7.0	3.4	0.1	0.1	10.4	X
97	17.6	5.8	68.0	25.0	50.4	19.2	43.0**	42.7**	69.6	XX
98	63.8	27.0	56.8	26.8	-7.0	-0.2	0.6	0.0	-7.2	(X)
99	21.8	13.4	68.2	42.4	46.4	29.0	13.7**	47.7**	75.4	XXX
100	36.0	14.6	73.0	32.4	37.0	17.8	8.0*	5.1*	54.8	XXX

* P less than 0.05 ($F_{.05} = 5.32$; degrees of freedom = 1 and 8)

** P less than 0.01 ($F_{.01} = 11.26$; degrees of freedom = 1 and 8)

comparisons; in addition they assisted in maintaining standard tests. Generally, however, reliance was placed on the observer's judgement of a response as gained from visual observation. At the close of each experiment, the strength of a response was recorded in one of several categories (or a fraction thereof) which are designated as follows:

Designation	Numerical equivalent	Description
0	0	No noticeable response.
(X)	0.5	An apparent sensing of the material; of doubtful biological significance.
X	1	A weak but positive response, usually including few of the components (surfacing, speeding, etc.)
XX	2	A moderate positive response, including most components.
XXX	3	A strong positive response, including all components.
XXXX	4	A very strong positive response, including all components; great excitement.

Often a reaction would be indicated by X(X) or XX(X), with numerical equivalents of 1-1/2 and 2-1/2 respectively.

Although admittedly subjective and not well-suited to statistical analysis, the foregoing measure is simpler and at times more meaningful than that dependent on the timing and counting alone. The two observers, by working together, attempted to standardize their judgement of the strength of a reaction. The observed reactions, measured in the above categories, are included in table 2 for the series of experiments analyzed in the preceding paragraphs. They may be compared with the summed time and count differences in the second to last column of the table. There is general agreement between the two measures in that the stronger observed reactions (XX and XXX) are associated with the greater differences between control and test quantitative data. However, there are also notable discrepancies as, for example, in comparing

No. 94 and No. 96: although the observed reaction is judged the same, X, the quantitative data diverge widely (although not significantly according to statistical tests). As will be indicated later, the observer's judgement of the strength of a reaction tended to change with time and with decreased reactivity of the fish. Thus a reaction which might be classed as X in August would likely be classed as XX or XXX in April.

Variation in Response

The strength of the response to an attractive substance, whether measured by the quantitative data or estimated by visual observation, varied greatly from test to test both within and between days. Throughout the mass of data there are numerous examples of this. For example in experiments Nos. 67, 72, 80, 97, 106, 111, 117, and 173, which were all conducted by one observer using the same material (250 ml. stock extract), the response varied from 1 to 3 (X to XXX) on the observational scale.

At times it was suspected that the variation in response was associated with either the frequency of testing or the accumulation of test products. These factors could readily influence the results obtained with fish in the tank, for it had both a small volume and a small rate of flow of salt water. Thus, following four experiments in which relatively large quantities of test substances were used (Nos. 59 to 62), there was no response to a relatively large volume (1,000 ml.) of stock extract (No. 63) in the fifth experiment of the day. After a rest period of 3 days in which no tests were conducted, much smaller volumes (250 ml.) of stock extract gave strong responses (Nos. 81 and 82). In the pond, at times there appeared to be a gradual increase in activity under control conditions and a gradual decrease in response to attractive substances when several experiments were conducted in succession during a day (fig. 5). This may be attributed to several possible causes including chance or random variation, an increasing concentration of materials in the pond, or an increasing threshold of response under repeated stimulation. When a response was protracted beyond the ordinary time limit, usually the observer would wait for 15 to 30 minutes before starting the next experiment to allow the fish to resume normal control activity. To some extent this would overcome the trends which are noted above.

In April 1953 a series of experiments (No. 313 et seq.) was undertaken to determine if there were differences in response to materials maintained at three different hydrogen ion concentrations (T_1--pH 2, T_2--pH 7, T_3--pH 10), but also to investigate

the variation in response within and between days. Both the experimental plan and the observational data are included in table 3. Two tunny were present in the pond throughout the 3-week period. Various difficulties were experienced which prevented the completion of all experiments in the series--either erratic behavior of the fish or other difficulties as listed in the footnotes to table 3.

The great variation in response is apparent from the data of table 3. For T_1 and T_2 it ranged from 1/2 to 4, and for T_3 from 1/2 to 2-1/2. A superficial analysis of that portion of the data comprising complete blocks (a.m. only) showed that none of the main effects (order of testing, days, and treatments) was significant. Order of testing contributed the largest mean square, days the next largest, and treatment the smallest. The last was less than the residual mean square. In these and other series of experiments often it has been noted that the first experiment of the day gives the strongest response: the fish are suddenly aroused from a quiescent condition and give a strong response relative to both the preceding control periods and the succeeding test periods. This contributes to the variation between means for order of testing (respectively 2.4, 1.3, and 1.3 for the observational data above). Variation in reactivity from day to day, apart from erratic behavior, has been noted repeatedly by both observers, and is believed real despite the non-significance of the data above. The reason is obscure--it is unlikely that it is related to the state of hunger of the fish; it has been suspected at times that it is related to the weather, tides, or turbidity, but these factors cannot be adequately investigated with the present data.

There seemed to be a decrease in the response and an increase in its variability between the summer of 1952 and the spring of 1953. Thus, in six pond experiments conducted with the same material (each equivalent to about 25 g. of tuna flesh) during August, the mean difference between timing for test and control periods was 32.6 seconds and the range was 11.2 to 52.2 seconds. In the test series conducted in April, in which the material was equivalent to about 350 g. of tuna flesh (14 times as much as before) treated in a different manner but one which did not significantly decrease its attractive properties, the mean difference between the timing for test and control periods was 7.1 seconds with a range of -13.0 to 61.8 seconds. The decreased response was reflected to only a slight extent in the observational data which averaged 1.9 for August and 1.7 for April; apparently there was a change in the observer's judgement of the absolute strength of a reaction. The reason for the decrease in response and the increase in its variability may be related to a decrease in the pond population from 13 to

Table 3.--Observational data on a series of experiments (No. 313, et seq.) conducted during April 1953 in Pond No. 5 (T_1--pH 2, T_2--pH 7, T_3--pH 10).

Week	Date	Observer	Treatment-Response						Mean
			Order - a.m.			Order - p.m.			
			1	2	3	1	2	3	
1	4/9	Tester	T_1--4	T_2--2	T_3--2	T_1--2	T_2--2	T_3--1	2.2
	4/11	van Weel	T_1--2-1/2	T_2--1	T_3--1/2	T_1--1-1/2	T_2--1/	T_3--1/	1.4
2	4/14	Tester	T_3--1-1/2	T_1--1	T_2--1	T_3--1	T_1--1/2	T_2--1/2	0.9
	4/18	van Weel	T_3--1/2	T_1--1	T_2--2-1/2	T_3--2/	T_1--1	T_2--1-1/2	1.3
3	4/21	Tester	T_2--2	T_3--3/	T_1--3/	T_2--3/	T_3--1/2	T_1--3	1.8
	4/25	van Weel	T_2--3-1/2	T_3--1-1/2	T_1--1/2	T_2--4	T_3--2-1/2	T_1--2-1/2	2.4

1/ Material lost because of leak in hose.

2/ Fish did not enter attraction area during test periods.

3/ Material not used; fish remained at one end of pond.

2 tunny. This decrease in the number of fish would, of course, decrease the absolute values of the quantitative data, particularly the counts, but it would not necessarily change the difference between control and test data. Apparently 13 fish gave a much greater response than 2 fish to the same amount of material, suggesting a "group" effect--a contagious excitement. On the other hand, the decrease in response may have been related to decrease in the "condition" of the fish and a declining interest in food substances during the winter months.

SOURCE AND PREPARATION OF TEST SUBSTANCES

Materials

The materials which were used are listed for each experiment in the appendix at the end of this report.

Because of its ready availability and also because it had given a positive response in experiments conducted by van Weel (1952), most of the preparations consisted of extracts of tuna flesh, including skipjack, tunny, yellowfin, and bigeye. In addition, extracts of tuna skin, blood, liver, ovaries, testes, and unsegregated viscera were used.

A few tests were conducted with extracts prepared from "white-fleshed" fish (as opposed to the red-fleshed tunas) when materials were available. They included "aholehole" (Kuhlia sandvicensis) flesh, jack (Caranx) flesh and blood, barracuda (Sphyraena barracuda) flesh, and wahoo (Acanthocybium solandri) flesh.

The tuna and other fish used in these preparations were either fresh-caught or fresh-frozen. They were obtained from our own fishing operations, from the Pacific Oceanic Fishery Investigations, or from the Hawaiian Tuna Packers, Ltd.

Beef blood obtained from a local slaughter house was used in comparison with fish blood.

Tuna cannery byproducts, including fish meal, stick-water (effluent), and various preserved digests of skipjack viscera made for other purposes by Mr. F. Jermann of Hawaiian Tuna Packers, Ltd., were tested. A herring reduction plant byproduct, "condensed herring solubles" prepared by B. C. Packers, Ltd., Vancouver, Canada, was also used.

As time permitted, tests were run on a variety of organic chemicals purchased from Nutritional Biochemicals Corporation and Eastman Kodak Company. A variety of aromatics used in perfume manufacture and food flavoring were supplied by courtesy of E. I. DuPont de Nemours and Company, Van Ameringen-Haebler, Inc., P. R. Dreyer, Inc., and Sindar Corporation. Miscellaneous substances which were tested included alleged commercial fish attractants and pineapple juice.

Preparation

It is not feasible to describe in detail the method of preparation of each substance which was tested. This has been summarized for each experiment and has been included in the appendix. General techniques and procedures, only, will be discussed here. Where necessary, details of special preparations will be included in later sections.

Simple extracts of fish flesh, skin, gonads, liver, etc. were prepared as follows: a quantity of material (usually about 100 g.) was weighed, cut into small pieces, placed in a Waring blendor with about 200 ml. of sea or tap water per 100 g. of material, and reduced to a homogeneous suspension of finely-divided particles over a 5-minute period. The material was then diluted to about 1 liter with water and was placed in a refrigerator (at about 1° or 2° C.) for several hours. The mixture would be used as such, or it would be separated into liquid and solid components by either filtering or centrifuging before testing.

A "stock extract" of skipjack flesh was prepared in bulk by grinding 9 pounds of flesh in a meat grinder, placing this in a milk can of 10 gallons capacity, adding tap or sea water to nearly fill the can, and placing the can and contents in the refrigerator. The solid material would settle from the liquid in a few hours. The clear, amber to cherry-red liquid (the exact color depending on the proportion of light to dark red meat in the sample) could be decanted from the surface. It was calculated that 1 liter of liquid contained the extract from about 100 g. of flesh. The stock extract could be maintained without putrefaction for 2 or 3 weeks in the refrigerator if the temperature was held just at or above the freezing point. The stock extract was used frequently as a standard in testing other substances.

In most of the experiments conducted during the winter and spring, 95-percent ethyl alcohol was used in the preliminary extraction of ground tuna flesh. The alcohol extract was amber to

cherry-red in color as in the case of the water extract. Alcohol served as both an extracting and a preserving agent; refrigeration of the extract was not necessary, although it was used occasionally.

More complex procedures were used in attempts to isolate and identify the attractive substance(s) in extracts of tuna flesh and viscera. These included fractionation of the extract into two or several components to determine, on pond testing, which still retained the attractive properties. In some preparations, a first separation into "protein" and "non-protein" factions was achieved by precipitation of the proteins and similar complex substances. Precipitation was accomplished in various ways, e.g., heating, boiling, saturation with sodium chloride or "salting out", adding tannic and/or phosphotungstic acid, adding the salts of heavy metals such as lead, barium, etc., as well as adding other chemicals to remove those used in precipitation. In other preparations the extract was separated into two fractions by dialysis to determine whether the attractant was comprised of a small molecule which would pass through a dialyzing membrane, or a large molecule which would not pass through a membrane. In still others the extract was divided into a "fatty" and a "non-fatty" fraction, and each was tested in the pond to determine if the attractant was a "fatty" or a "non-fatty" substance. Fractionation was accomplished by removing the fatty acids and similar compounds by extraction with petroleum ether and/or acetone. Other procedures were aimed at determining whether the attractive substance consisted of a positive or negative ion. In these, fractionation was achieved by passing the extract through anion and/or cation exchange resin columns giving two portions, that adsorbed by the column (subsequently removed by elution or washing) and that not adsorbed by the column (the filtrate). In addition, columns of activated charcoal and activated alumina were used to see if they would adsorb the attractive substance.

In many of the preparations, several of the foregoing fractionation procedures were used in succession, each successive portion being tested and being either accepted or rejected for further fractionation depending on whether pond tests indicated that it did or did not include the attractive substance. Several of the procedures were used even though they did not remove the attractive substance; they did remove non-active substances and thus constituted a step in the purification of the attractant. The attractant, when purified as completely as possible by the successive removal of non-attractive substances, was then subject to basic chemical tests in an attempt to determine its basic components and chemical structure.

Miscellaneous chemical compounds were prepared for testing by dissolving in tap water or in weak alcohol solutions, or in

the case of fatty or oily substances, by dispersing in water with a dispersing agent "Tergitol". If the latter was not effective, the mixture was forced through an emulsifying apparatus to insure its complete dispersion.

RESPONSE TO SIMPLE EXTRACTS OF TUNA AND OTHER FISH

Flesh Extracts of Tuna

There are many experiments to show that in both the tank and the pond the tunny (and to a lesser extent, the yellowfin) gave a positive response to clear, aqueous extracts of skipjack flesh (Nos. 1, 8, 10, 13, 20, 50, 54, 64, 65, 66, 67, 72, 80, 81, 82, 87, 93, 97, 106, 111, 112, 117, 118, 122, 128, 135, 173, 199, 207), yellowfin flesh (Nos. 14, 18, 26), tunny flesh (No. 27) and bigeye flesh (Nos. 239, 252, 259). To these might be added a large number of experiments using either water or alcohol for extraction in which the materials were subjected to further treatment and which in many cases gave a positive response. In only a few experiments with simple extracts were negative results obtained when positive results were expected. In tank experiment No. 16, the fish had been fed 2 hours previously and were not responsive to skipjack flesh extract. In tank experiment No. 63 there was no response, due probably, as has been mentioned previously, to the dulling produced by an accumulation of test products in the tank with too frequent testing. In pond experiment No. 336, the fish failed to enter the attraction area during the 15-minute test period. In a few experiments, of course, the response was uncertain or slight (X).

The nature of the response to extracts of tuna flesh has already been described. It is a complex reaction, consisting primarily of an urge to feed, which is manifested by speeding, surfacing, circling, snapping at objects, together with a return to the area of stimulation.

It is presumed that in tuna flesh there is present a substance (or substances) which, when extracted and presented to the tunny, can be perceived through the fish's sense of smell or taste. The substance which, when smelled or tasted, promotes the urge to feed is henceforth called the "attractant." Unfortunately, as already pointed out, the response varies greatly in strength or manifestation between similar experiments. It appears to be influenced by many uncontrolled and perhaps uncontrollable factors.

One experiment (No. 112) is described in detail, for it differed from the others in method and purpose. Three liters of stock

extract was introduced into the pond, with the pump in operation, by slowly siphoning it into the funnel over a 48-minute period. This established a gradient of attractant from the outlet to beyond the attraction area. Timing, only, was attempted and this consisted of the time out of 3 minutes spent in the first 1/10th of the pond, i.e. the area between the seaward gates and the first string (fig. 4), not including the shallows. The results are shown in figure 7.

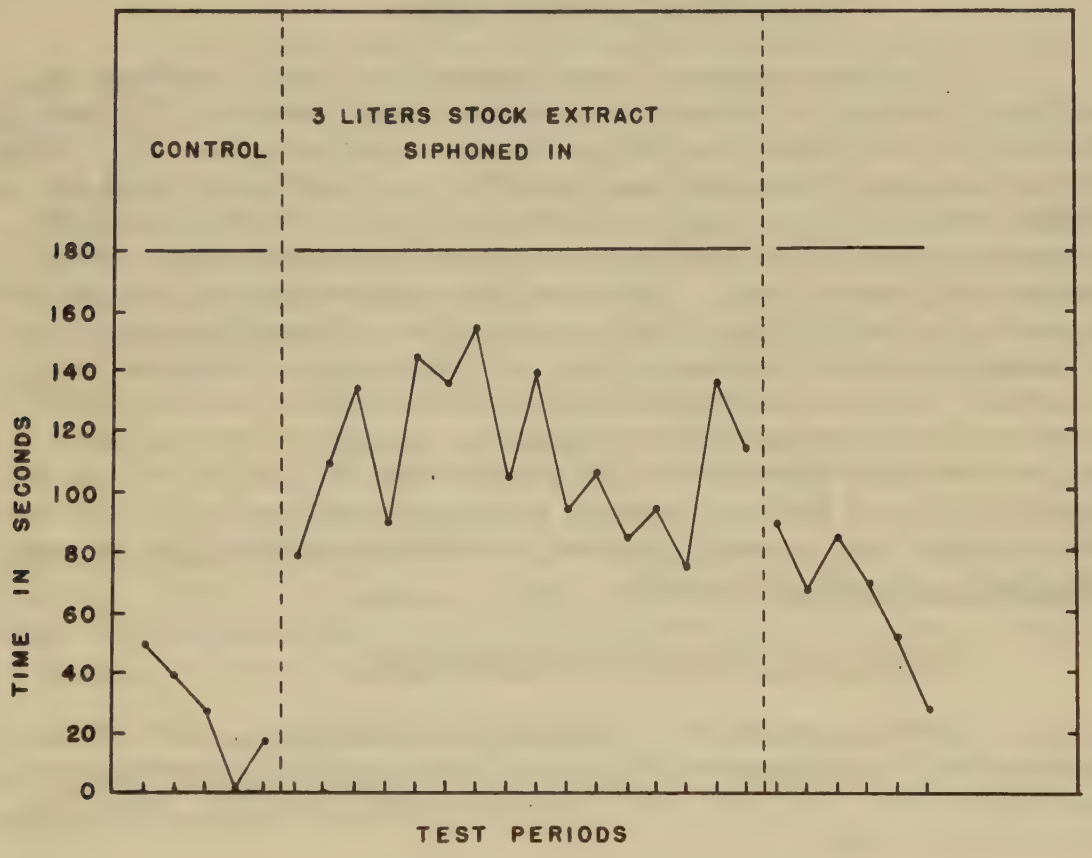


Fig. 7.--Time (seconds) out of successive 3-minute periods in pond experiment No. 112, in which a gradient of stock extract of skipjack flesh was established for 48 minutes.

The material was sensed very quickly by the tunny and produced a typical response, with all components, in the vicinity of the outlet. The response was maintained during the entire time necessary to siphon in the material. A few fish would leave the area and dash down the pond, but they would invariably return to the area of greatest concentration. After all the material had been siphoned in, the response quickly decreased until finally, after about 18 minutes, normal swimming was resumed.

Flesh Extracts of Other Fish

Positive responses were obtained not only to extracts of tuna flesh, but also to extracts of aholehole flesh (Nos. 49, 99, and 105), jack or ulua flesh (No. 96), and barracuda flesh (No. 98). There was no response to wahoo or ono flesh (No. 6), but the fish were not behaving well in this early tank experiment. It is evident that an attractant is present in at least some of the white-fleshed fish, as well as in the red-fleshed tuna. We gained the impression that the response was generally less in these flesh extracts than in those from tuna, but this cannot be proved because of the few experiments conducted with the former, variation in the quantity of material used, and the great variability in the strength of the response. In most of the comparisons the response appeared less (compare Nos. 49 and 50, 96 and 97, 97 and 98, 105 and 106) but in one it was greater (97 and 99). Van Weel (1952) obtained only a weak response to white-fleshed anchovy or nehu (Stolephorus purpureus) extract.

Extracts of Tissues Other than Flesh

Strong responses, ranging from XXXX to X, were obtained with extracts of unsegregated skipjack viscera treated in several different ways (Nos. 137, 139, 141, 145, 146, 219, 220, 221, 236, 264, 279, 352, 353, 356).

Excellent responses were also obtained with extracts of skipjack blood (Nos. 43, 70, 95), yellowfin blood (No. 23), and tunny blood (No. 30). Jack or ulua blood (No. 94) gave a positive though weaker response. In contrast to these results beef blood, either whole (Nos. 55 and 74) or the plasma portion (No. 61), was not attractive. In fact, whole beef blood, of a deep red color, was avoided by the tunny in both the tank and the pond experiments--a repellent effect which was probably visual in origin.

Excellent responses were obtained with extracts of yellowfin liver (No. 25) and tunny liver (No. 28).

Extracts of skipjack ovaries (No. 3) and yellowfin testes (Nos. 4, 17) were not attractive. However, whole extracts were used and the fish may have been frightened by the murky cloud which they produced in the water.

Unfiltered extracts of yellowfin skin (No. 24) and tunny skin (No. 29) also formed a dark cloud in the water and were more repellent than attractive. An almost clear centrifuged extract of skipjack skin (No. 48) gave a weak positive response.

It appears that the attractant is present not only in tuna flesh, but also in other parts of the fish. Again, however, it is difficult to come to any firm conclusion as to the relative strength of the response, and thus the relative concentration of the attractant in the various parts. In general the viscera extracts appeared to be at least as attractive as the flesh extracts, and possibly slightly more attractive. In two series of experiments designed to compare extracts of blood, liver, skin, and flesh (Nos. 23 to 26, Nos. 27 to 30), extracts of blood and liver appeared to be more attractive than extracts of flesh, and extracts of skin appeared to be less attractive.

Cannery Byproducts: Preservation

Good (XX) responses were obtained with extracts of tuna fish meal, both boiled (No. 119) and not boiled (No. 120) during extraction. Excellent responses (XXX) were obtained with tuna stickwater both by itself (No. 217) or mixed with fluorescein dye to trace its dispersion in the pond (No. 222).

"Condensed herring solubles," a product derived from the stickwater of a herring reduction plant (B. C. Packers, Ltd.) was not sensed by the fish (Nos. 328 and 329).

Several experiments were run on skipjack viscera preparations submitted by Hawaiian Tuna Packers, Ltd., with excellent results (XX to XXXX). In all preparations the viscera from the cannery were first ground in a meat grinder. In some they were preserved in 2- or 3-percent sulphur dioxide and self-digested, producing a particulate portion which settled to the bottom of the container and a supernatant dark brown liquid (Nos. 219, 221, 290, etc.). In others, the ground viscera were steamed at 20 pounds pressure and preserved in 3-percent phosphoric acid, again producing a solid and a supernatant yellow liquid (No. 220). A ground viscera preparation preserved in 2-percent sodium bisulphite and digested with pepsin gave little evidence of attraction in three tests (Nos. 258, 260, and 263). Apparently loss of attraction was due to something associated

with pepsin digestion rather than to the use of sodium bisulphite, for this preservative was used successfully in other preparations (Nos. 264, 279, etc.).

The above results, indicating that the attractive qualities are not lost when the materials are preserved in chemicals, are in accord with those obtained with our own preparations which were tested at an earlier date. Skipjack viscera extracted with water and treated with 2-percent sulphur dioxide (Nos. 140, 141), with 2-percent sulphuric acid (No. 144), or with 2-percent phosphoric acid (No. 145) were still attractive to the fish even after being kept at room temperature for several days.

RESPONSE TO CHEMICAL COMPOUNDS AND MISCELLANEOUS MATERIALS

As time permitted, a variety of chemical compounds ranging from amino acids to proteins and including vitamins and various miscellaneous aromatics were tested as follows:

Arginine (No. 32, 103)

l-Asparagine (No. 102)

Creatine (No. 109)

l-Glutamic acid (No. 191)

Glycyl-glycine (No. 127)

l-Histidine monohydrochloride (No. 192)

dl-Isoleucine (No. 34)

l-Leucine (No. 44)

Methionine (No. 157)

dl-Norleucine (No. 45)

dl-Phenylalanine (No. 31)

dl-Serine (Nos. 51, 56, 101, 104, 108)

l-Taurine (No. 42)

Acetyl tryptophane (No. 176)

l-Tyrosine (No. 33)

dl-Valine (No. 41)

Isoeugenol (No. 37)

Nucleic acid (No. 110)

Histamine diphosphate (No. 198)

Adenosine (No. 242)

Adenosine triphosphate, disodium salt (Nos. 243, 254, 271)

Adenosine diphosphate, barium salt (Nos. 245, 255, 270)

Adenylic acid (No. 244)

Trimethylamine oxide (No. 188)

Animal lecithin (No. 129)

Albumen (No. 62)

Peptone "I" (No. 189)

Vitamin B_{12} (No. 166)

Acetyl choline chloride (No. 192)

Pregnenolone (No. 132)

Ambergris (No. 131)

Anethol (Nos. 22, 71)

Phenyl acetic acid (No. 251)

Alpha ionone (No. 158)

Alamask (No. 172)

Furfuryl mercaptan (Nos. 231, 241)

Coumarin (No. 249)

Methyl anthranilate (No. 250)

Meat flavor (No. 253)

Pineapple juice (No. 168)

Eosin B (No. 36)

Methylene Blue (No. 38)

None of the above substances gave a positive response which could be verified. In some, however, there appeared to be a sensing of the substance as will be discussed below.

Among the amino acids, serine appeared to give a weak positive response in one tank experiment (No. 51) and a very weak positive response in another (No. 56). In three pond experiments, there appeared to be a sensing of the material in one (No. 101) but not in two others (Nos. 104 and 108). On the same day on which the fish appeared to sense the serine in the pond (No. 101) they also appeared to sense asparagine (No. 102) and arginine (No. 103), suggesting that they were in a particularly responsive condition. On another occasion they appeared to sense methionine (No. 157). Apparently the fish were aware of the presence of the substances but the slight reaction seemed to be one of curiosity rather than attraction: certainly it lacked the features of a typical response.

This same sensing of material was noted in the case of certain strong-smelling aromatics, e.g., anethol (No. 22), "meat flavor" (No. 253), and particularly furfuryl mercaptan (Nos. 231 and 241). The first substance, which has a licorice smell, is the active ingredient of oil of anise, a reputed fish attractant; the second is a strong, spicy, pleasant-smelling artificial flavor; the third is a very foul-smelling chemical of great potency. A similar response was noted with odorless vitamins, B_{12} (No. 166) and acetyl choline chloride (No. 192), and also with ordinary pineapple juice (No. 168). Again, however, the response seemed to be one of curiosity.

The series of experiments with adenosine, adenosine compounds, and adenylic acid was conducted in an attempt to determine if the attractive substance was one which is present in mammalian muscle. That it might be was suggested by chemical tests of the attractant (see later) which showed the presence of phosphorus and the amide

link. The substances were dissolved in sea water immediately before testing to avoid hydrolysis. The barium salt of adenylic diphosphate was dissolved in 0.1 N hydrochloric acid and the barium was precipitated with 6N sulphuric acid to have it present as free adenosine diphosphate acid, rather than as a barium salt (there had been indications that barium tended to destroy the attractive material). It was presumed that the pH of seawater would assure the proper molecular form of the sodium salt of adenosine triphosphate. First tests indicated a sensing and an attraction of the tunny to both adenosine triphosphate (No. 243) and diphosphate (No. 245) but not to adenosine (No. 242) nor to adenylic acid (No. 244). However, later tests with both the triphosphate (Nos. 254 and 271) and the diphosphate (Nos. 255 and 270) gave negative results.

The two dyes, eosin red (No. 36) and methylene blue (No. 38), were visually repellent to the fish. This same reaction, an avoidance, was occasionally noted with strong solutions of the greenish fluorescein.

In addition to the substances listed above, two alleged fish attractants were tested, one known as "Slik" which smelled of cod liver oil, and the other an unnamed "fish lure" which smelled of tuna or herring viscera extract. There was a slight sensing of the first (No. 174) and a weak but positive reaction to the second (No. 349).

ATTEMPTS AT ISOLATION AND IDENTIFICATION OF THE ATTRACTANT (S)

The attractant(s) was initially segregated from the flesh or other parts of the fish by virtue of its solubility in water. Thus, on centrifuging, the aqueous extract of finely-divided tuna flesh formed a clear amber to reddish colored solution which usually caused a strong positive response (Nos. 1, 8, 10, and numerous other experiments in both tank and pond). The residue from centrifuging was either attractive (No. 15) or not (No. 2), depending on either the thoroughness of the extraction or the part played by vision in the case of the murky mixture.

In early tank experiments, boiling of tuna flesh with water, thus precipitating the proteins and similar compounds, appeared to remove or destroy the attractant. The clear filtrate from the boiled flesh failed to elicit a response (Nos. 5, 9, 11, 12, 21) as was also found previously by van Weel (1952). However, as will be discussed later, this was not the case with boiled extract of flesh.

Dialysis, using a cellophane tube (1-5/8 inch diameter), was next tried in an attempt to segregate the attractant, using clear

aqueous extracts of skipjack flesh (several experiments) or tunny blood (one experiment). In all cases the dialyzate gave a positive response when tested (Nos. 39, 46, 53, 57, 90, 118, 123), showing that the attractant consists (in part, at least) of a small molecule, rather than a large molecule such as a protein. In no case, however, was complete separation attained by dialysis. In most of the experiments the residue (portion remaining in the tube) gave a stronger response than the dialyzate (compare Nos. 39 and 40; 52 and 53; 57 and 58; 90 and 91) despite several changes of the distilled water surrounding the tube.

Precipitation of the proteins by dilute (10-percent) hydrochloric acid was next attempted. After the centrifugate of an aqueous extract of skipjack flesh was treated with acid and centrifuged, both the neutralized centrifugate (No. 68) and the neutralized residue (No. 69) gave positive responses, showing incomplete separation of the attractant.

As the above results indicated that the attractant was not a protein, a series of pond experiments was undertaken in which the proteins were precipitated by heating. On heating the centrifuged aqueous extract of skipjack flesh to 69° C., a precipitate appeared. When this was removed by centrifuging, the centrifugate was still attractive (No. 76). When the centrifuged aqueous extract was boiled for 30 minutes, resulting in a heavy precipitate, both the filtrate in one experiment (No. 77) and the centrifugate in another (No. 78) were still attractive to the fish. However, separation was not complete, as is shown by the weaker positive responses obtained with the washed residue from centrifuging (compare Nos. 75 and 76; Nos. 78 and 79).

It is uncertain why the filtered extract of boiled tuna flesh failed to give a positive response in the earlier tank experiments Nos. 5, 9, 11, 12, and 21). Although the behavior of the fish in the tank was not too satisfactory, it seems certain that had a response occurred, it would have been noted or measured in one of the five experiments. It is unlikely that the attractant was destroyed by boiling in view of the results reported in the preceding paragraphs and others to be presented later. There remains the possibility that the attractant was precipitated with the soluble proteins or otherwise adsorbed with the insoluble proteins during the process of boiling the flesh; the residue, which may have contained the attractant, was not tested in the five experiments conducted with the boiled flesh.

Chemical tests were run on the clear centrifugate obtained after boiling of the extract. The Biuret test was positive, showing the presence of the amide link. The Xanthoproteic test was positive,

indicating the presence of the benzene ring. No positive test for protein was given on layering the solution over concentrated nitric acid. The lead test for sulphur was negative.

A series of experiments was conducted to compare the effects of precipitation by boiling and precipitation by salting out of the proteins on the attractiveness of the filtrate. The procedure and results are depicted in figure 8. Clear stock extract was boiled for 15 minutes and centrifuged; the centrifugate was divided into three equal parts, the first of which was tested (No. 85), the second of which was saturated with sodium chloride, centrifuged, and both centrifugate (Nos. 83, 86) and residue (No. 88) tested, and the third of which was boiled for an additional hour, centrifuged, and the centrifugate tested (No. 84). Strong responses were obtained with all tests except that with the residue (No. 88). The attractant was not removed with precipitation in either boiling or salting. Apparently fractionation was complete when both boiling and salting were used (compare Nos. 83 and 86 with No. 88).

The boiling and salting procedure was carried one step farther in two additional experiments. The centrifugate, after boiling and salting, was boiled to a residue. When re-dissolved in seawater, a positive response was obtained (Nos. 113, 121).

The possibility of precipitating or destroying the attractive substance by heat after it had been segregated in a relatively pure (protein-free) state by dialysis was next investigated. One liter of the dialyzate from a stock extract was boiled to 10 ml. and tested, producing a very strong response (No. 118). The residue from dialysis was boiled to a gummy residue, producing weaker but positive responses with both the 95-percent alcohol soluble portions and the alcohol insoluble portions (Nos. 114, 115, 116). In a second series, the dialyzate was boiled to dryness and a strong positive response was obtained with the portion of the residue which was soluble in absolute ethyl alcohol (No. 123). The solubility in absolute alcohol was confirmed in later experiments (Nos. 201, 281).

Thus the attractant is a substance comprised of a small molecule which is (at least, partly) soluble in absolute ethyl alcohol. This property rules out most of the known amino acids (except methionine and proline). However, that part of the attractant or attractants was insoluble in alcohol yet soluble in water is indicated by several experiments in which the alcohol-insoluble, water-soluble portion gave a positive response (Nos. 115, 116, 124, 183).

Because of the time required for dialysis and the incomplete separation that was achieved, attention was directed at

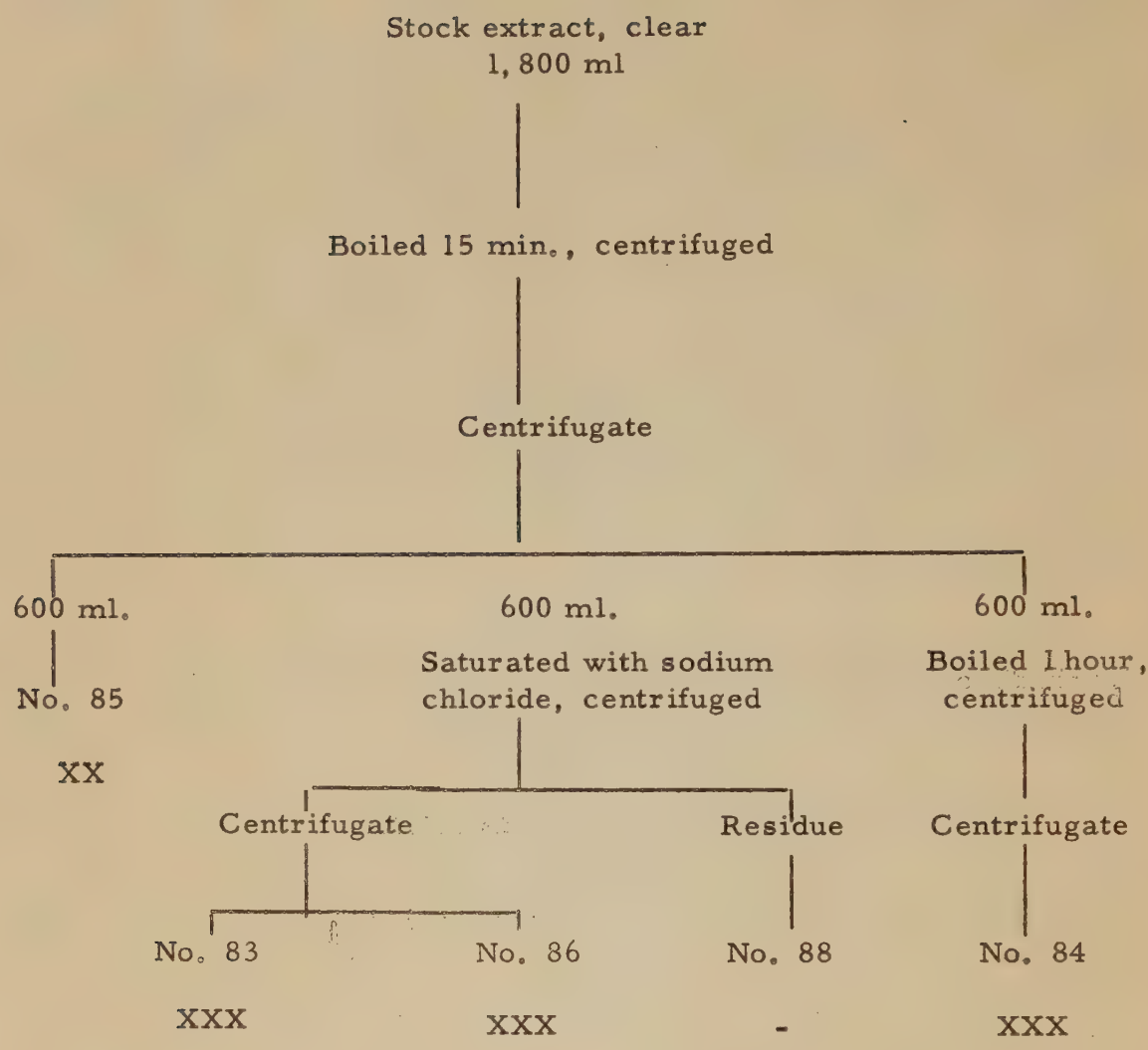


Figure 8.--Fractionation of stock extract of skipjack flesh: procedure and results in experiments Nos. 83 to 86, and 88.

the possibility of adsorption on various media. In one experiment, stock extract was concentrated by boiling, filtered, boiled with activated carbon, filtered, evaporated to a gummy residue, re-dissolved and tested, giving a positive response (No. 125). In another, the same procedure was followed except that after boiling with activated carbon the filtrate was treated with activated alumina, again with a positive response (No. 130). Similar results were obtained with anion (No. 133) and cation (No. 134) exchange resins. Apparently the attractant was not (completely) adsorbed on any of these media.

At this time (September 1952) the chemical work was undertaken at the University of Hawaii rather than the Hawaii Marine Laboratory; the tests, of course, were continued on the fish in Pond No. 5 at Coconut Island. To obviate the need for refrigeration and to facilitate condensation, extracts were prepared with 95-percent ethyl alcohol instead of water. The actual strength of the alcohol in the extract was less than 95-percent, depending on the quantity of water in the flesh and the relative quantities of flesh and alcohol which were used, but it was always strong enough to act as a preservative at room temperature. Numerous experiments showed that this alcohol extract promoted a strong positive response (Nos. 147, 163, 180, 235, etc.), whereas there was no response to alcohol alone (No. 19). However, complete extraction of the attractive substance with alcohol was difficult to attain. In two experiments, the flesh was treated with 95-percent alcohol for several hours and the alcohol, containing some of the attractant, was filtered off and used for further fractionation. The flesh was again treated with alcohol, and the alcohol filtered off and used. The flesh was then treated with water and the filtrate was tested. It still gave a strong response (Nos. 164 and 169) showing that the attractant had not been completely removed by the previous two alcohol extractions. To see if complete separation could be achieved with alcohol extraction, flesh was extracted twice with 95-percent alcohol as above. It was then further extracted with alcohol for 14 hours in the Soxhlet apparatus. The alcohol was then removed, and the flesh was extracted for an additional 12 hours with distilled water. The centrifuged water extract gave only a slight response (No. 17), indicating that almost complete extraction of the attractant had been achieved by the previous alcohol treatments.

Further attention was directed at fractionation of the alcohol extract by use of various adsorption media. To improve the technique, columns of the adsorbing media were prepared, through which the extract was allowed to filter slowly. Two portions were obtained, the filtrate or material which filtered through the column without being adsorbed, and the eluate or portion which was adsorbed

on the column and which was eluted or washed from it with various solutions such as water, weak acids, weak bases, alcohol, acetone, etc.

In a series of experiments with columns of activated alumina, zeolite, and activated carbon (Nos. 149 to 154) positive responses were obtained from both the filtrate and (water) eluate of the alcohol extract of skipjack flesh. However, the attractant appeared to be adsorbed most on activated alumina (No. 150) and least on activated carbon (No. 153). Adsorption on activated alumina was obtained in two additional experiments (Nos. 156 and 165), but complete separation of the attractant was not achieved (Nos. 155 and 162). Attempts at fractionation with alumina were abandoned in favor of anion and cation exchange columns.

Small white crystals which appeared in the (acetone) eluate from the activated carbon column, when dissolved in water, appeared to cause a positive response (No. 159). Further preparations gave no response (No. 167) and a doubtful response (No. 171). It was concluded that the positive responses were apparent rather than real and that the white crystals were not attractive. The attractive substance, for the most part, appeared in the filtrate from the carbon column. Thus either mixing or boiling activated carbon with the extract or passing the extract through a carbon column would remove certain inactive substances and constitute a stage of purification. This procedure was followed in some of the later preparations.

Another step in purification was the removal of fatty acids and related compounds which were soluble in petroleum ether or acetone. Van Weel (1952) found that the attractant was not soluble in petroleum ether. This finding was verified (Nos. 18, 142, 175, 177, etc.). It was also found that the attractant was not removed by extraction with acetone (No. 142).

Still another step in purification was the removal of inactive proteins by precipitation with tannic acid (No. 215), lead acetate (No. 197; Nos. 200 to 206; etc.), or barium acetate (Nos. 211, 212, 225) and the removal of purines and similar compounds as silver salts (Nos. 183, 184) or with phosphotungstic acid (No. 193, etc.).

Some of the above steps in purification either preceded or succeeded fractionation by adsorption on anion or cation exchange resins. Many different experiments, employing one to several successive stages of purification, were conducted. Two typical series only will be discussed to illustrate the procedure and results. Others may be followed from data given in the Appendix.

The first, a relatively simple series designed to determine whether the charcoal-purified, "fat-free" attractant was adsorbed on ion exchange resin columns, is illustrated in figure 9. An alcohol extract of skipjack flesh was passed through a column of activated charcoal; the filtrate was evaporated to dryness and extracted with petroleum ether and the residue was dissolved in alcohol and divided into two portions. One portion was passed through a Duolite C-3 cation exchange resin column and the filtrate collected and tested (No. 178); the column was then eluted with 5-percent sulphuric acid and the eluate was collected and tested (No. 181). The other portion was passed through a Duolite A-3 anion exchange resin column, and the filtrate was collected and tested (No. 179); the column was then eluted with 5-percent sodium hydroxide and the eluate was collected and tested (No. 182). In this particular series all tests were positive, but the eluate from the anion column (No. 182) appeared to give the strongest response.

The above results indicated that a further investigation of the adsorption on an anion column would be profitable, particularly to see (a) if complete adsorption of the attractant could be attained, (b) to see if the adsorbed material was completely removed by a water eluate, or if additional adsorbed material could be recovered with either a weak base or a weak acid and (c) to examine the solubility of the attractant in absolute alcohol. The series is illustrated in figure 10. A clear alcohol extract of tuna flesh was treated with lead acetate to remove proteins, filtered, and the filtrate was extracted with petroleum ether; it was then treated with hydrogen sulphide gas to precipitate the lead and excess gas was removed in vacuum. The filtrate was passed twice through a Duolite A-3 anion exchange resin column and the filtrate collected and tested (No. 200). The column was then eluted, first with water, next with a weak acid, and finally with a weak base. The water eluate was evaporated to dryness and dissolved in absolute alcohol (No. 201); the residue was dissolved in water (No. 202). The base eluate was similarly treated, giving an alcohol soluble portion (No. 204) and an alcohol-insoluble, water-soluble portion (No. 205). A positive response was obtained only with the alcohol soluble portion of the water eluate. In this series it appears that complete separation of the attractant was achieved by use of the anion exchange column and that the adsorbed attractant was completely removed by the water eluate.

In these and other experiments conducted during the autumn, it appeared that the attractant, purified to a varying extent, was not adsorbed on a cation exchange column, for positive responses were obtained with the filtrate (Nos. 142, 178, 216, 225) rather than the eluate. Only two tests were performed with the latter, however, one

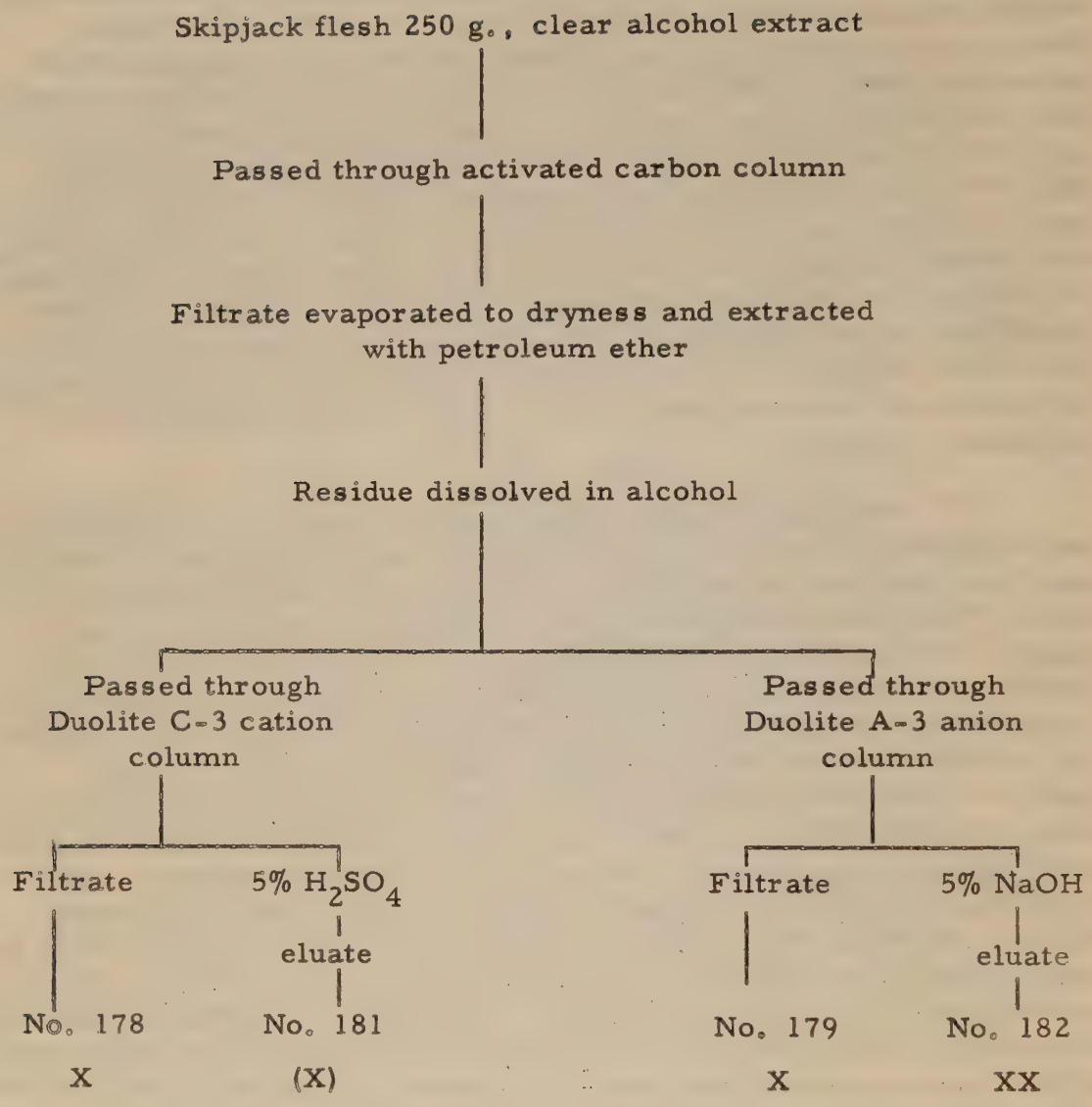
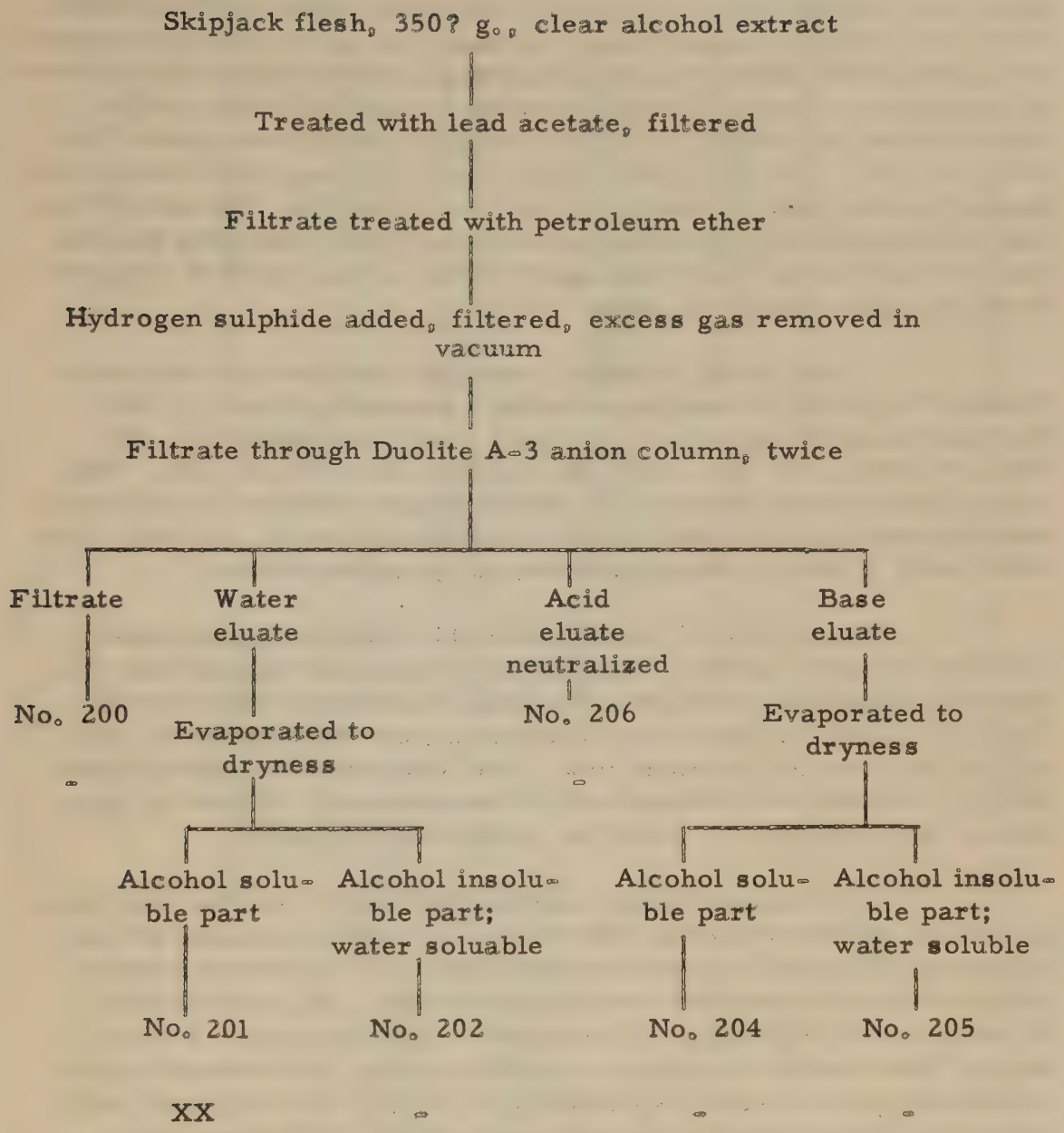


Figure 9.--Fractionation of an alcohol extract of skipjack flesh: procedure and results in experiments Nos. 178, 179, 181, and 182.



XX

Figure 10.--Fractionation of an alcohol extract of skipjack flesh: procedure and results in experiments Nos. 200 to 202, and 204 to 206.

giving no response (No. 143) and the second giving a slight response (No. 181). More attention was directed to the anion exchange column, for it seemed to adsorb the attractant in several experiments as shown by moderate to strong (X and XX) positive responses with a water, dilute sodium hydroxide, or dilute ammonium hydroxide elutant (Nos. 182, 185, 193, 194, 201, 208, 213). Up to this stage of the study, the eluate failed to give a response in only two tests (No. 196 and 223) in which a response was expected on the basis of the preceding results. The filtrate, on the other hand, gave either a weak positive response or no response (Nos. 179, 200).

It was clear, however, that the attractant was held very "loosely" on the anion exchange column, appearing mostly in the first portion of the water or weak base eluate (compare Nos. 185, 186, 187; Nos. 208, 209, and 210; 213,214; 246, 247 and 248). The weak affinity (possibly not a true ion exchange) explains the failure of complete separation in some of the above experiments, and may have contributed to certain inconsistencies to be reported later.

Chemical tests were run on the attractant in its purest form yet attained, namely, that portion of the water eluate from the anion exchange column which was soluble in absolute ethyl alcohol (No. 201). Both the Biuret and Ninhydrin tests were positive, showing the presence of the amide link. The Xanthoproteic reaction was positive, showing the presence of the benzene ring. A test for phosphorus was positive, but a test for sulphur was negative.

In subsequent experiments which were run from the latter part of December on through the winter, the results became erratic. There was difficulty in obtaining meaningful and consistent results with the various chemicals used in purification, and also with the anion and cation exchange columns used in fractionation. For example, when barium acetate was substituted for lead acetate to precipitate the proteins and the filtrate was treated with sulphuric acid rather than hydrogen sulphide gas to precipitate the barium as a sulphate, no response was obtained (No. 211) . Also there was no response for the solution that resulted when the protein precipitate of No. 211 was dissolved with nitric acid and the barium precipitated as a sulphate and filtered off (No. 212). The loss of activity, if real, may have been due to either destruction or CO precipitation of the attractant by the barium ion or by the sulphuric or nitric acid. That the barium ion was not responsible is shown by the series of experiments depicted in figure 11. An alcohol extract of skipjack flesh was acidified and treated with phosphotungstic acid to precipitate the purines. Barium acetate (in one experiment) and barium chloride (in another) was used to precipitate the phosphotungstic acid and the

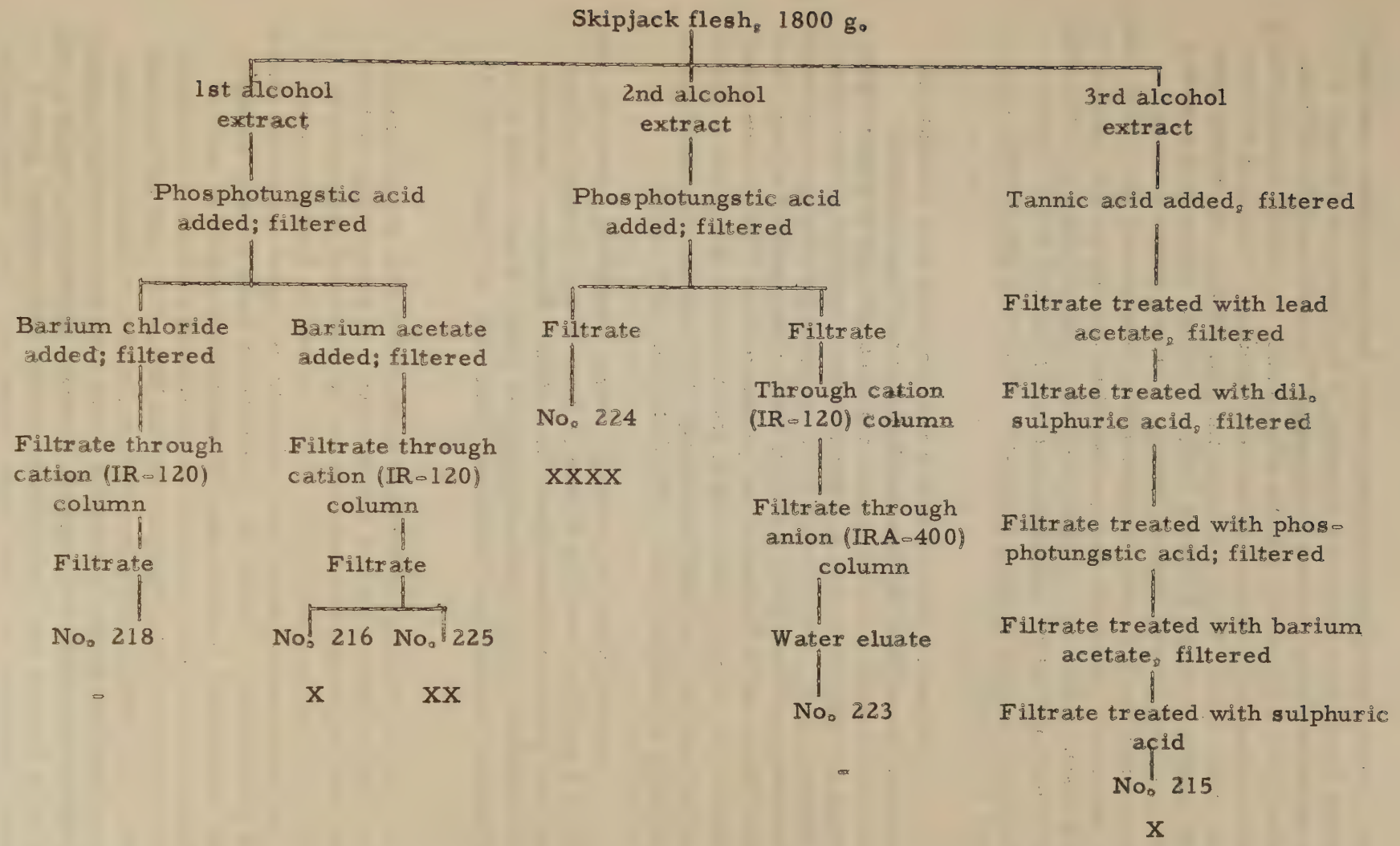


Figure 11.--Fractionation of alcohol extracts of skipjack flesh: procedure and results in experiments Nos. 215, 216, 218, and 223 to 225.

proteins. There was a good response when barium acetate was used, the barium ion was removed by passage of the material through a strong cation exchange column (Amberlite IR-120), and the filtrate was tested (No. 216, 225). On the other hand, strangely enough there was no response with exactly the same treatment, but using barium chloride (No. 218). When the material treated with phosphotungstic acid was passed through a strong cation exchange column (Amberlite IR-120), the filtrate was passed through a strong anion exchange column (Amberlite IRA-400), and the eluate was tested (No. 223), there was no response, although one was expected judging by the results of previous tests. However, a weak response was obtained with the final filtrate from a series of precipitations involving the use of both the barium ion and sulphuric acid (No. 215), following the method of Suzuki et al. (1909).

Inconsistent or negative results were also obtained in attempting to repeat the various purification and fractionation techniques discussed above on a self-digested extract of ground skipjack viscera, preserved with sulphur dioxide, supplied by Hawaiian Tuna Packers, Ltd. The extract itself promoted a strong response in some experiments (No. 219), but only weak responses in others (Nos. 229, 230). It could be cooked under pressure (No. 236) or extracted with alcohol (No. 235) without loss of its attractive properties, but it could not be carried successfully through the various precipitation nor ion adsorption processes (Nos. 232, 233, 234, 237, 238).

As skipjack flesh was no longer available, attention was directed at a study of extracts of bigeye tuna flesh. Simple alcohol extracts gave good responses (Nos. 239, 240). However, weak or negative responses were obtained when the purified materials were passed through ion exchange columns (Nos. 246, 247, 248, 256, 257, 265).

The negative results in these tests may have been due either to loss or destruction of the attractant at some stage of fractionation, or they may have been due to the moribund condition of the fish prior to a January mortality. To examine the former possibility a series of experiments with extracts of bigeye flesh was undertaken as illustrated in figure 12. The filtrate from alcohol extraction was treated with lead acetate to remove the proteins, and filtered; the filtrate was treated with sodium chloride to precipitate the lead as lead chloride, and was tested (No. 266) with an excellent response. The filtrate from the sodium chloride treatment, when boiled, gave only a slight response. Some loss may have been associated with boiling after treatment with lead, although a repeat of the procedure by heating on a water bath, rather than boiling, gave a very strong response in a later experiment (No. 273). When part of

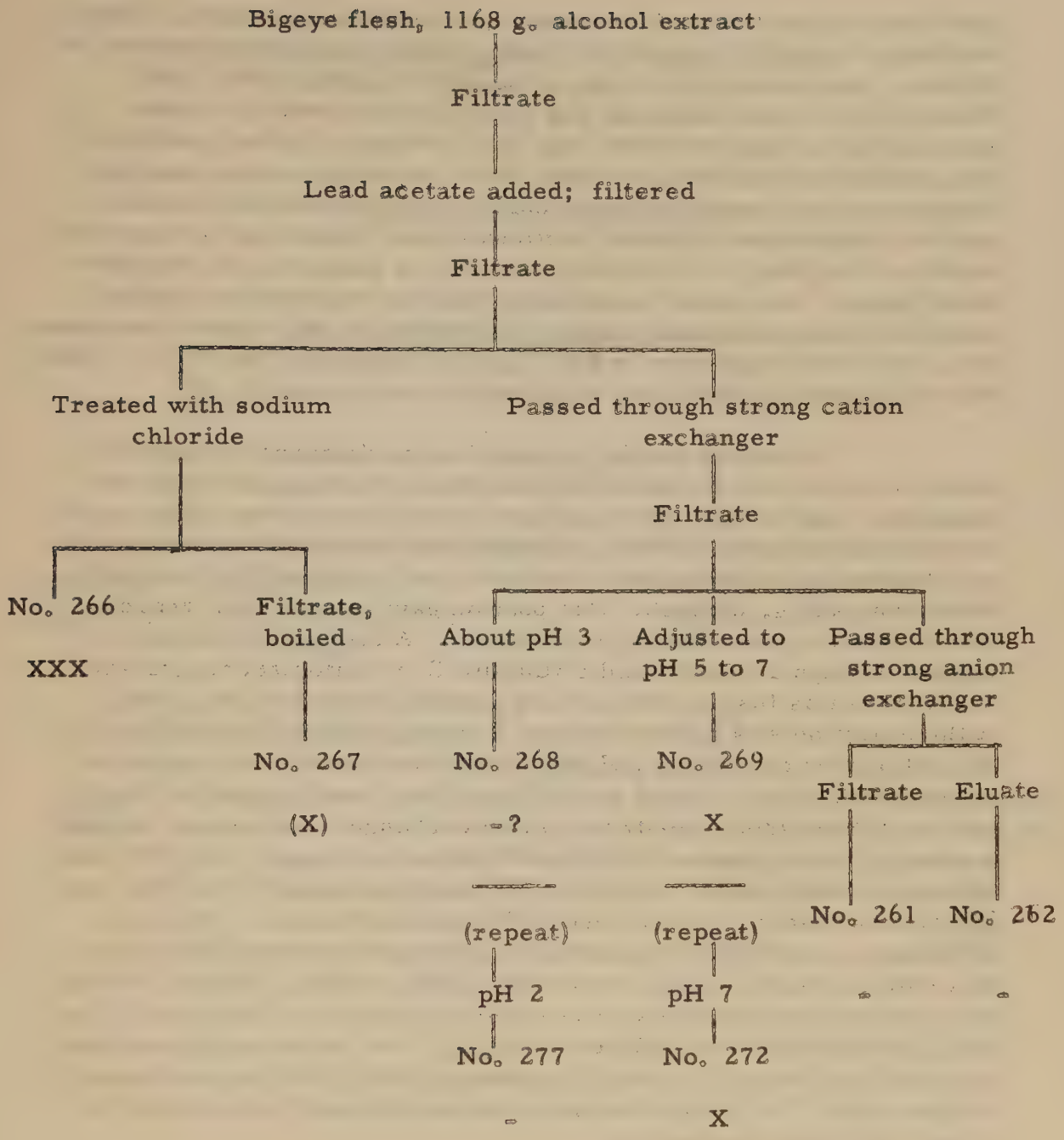


Figure 12.--Fractionation of an alcohol extract of bigeye flesh: procedure and results in experiments Nos. 261, 262, 266 to 269, 272, and 277.

the filtrate from the lead treatment was put through a strong cation exchange column to remove the lead, and the unadsorbed portion, or filtrate, was tested, there was no positive response (No. 268). When the pH of this material was adjusted from about 3 to near neutral, there was attraction (No. 269). When a further portion of the unadsorbed material from the cation exchange column was put through a strong anion exchange column and the adsorbed (eluate) and the unadsorbed (filtrate) portions tested, there was no response to either one (Nos. 261 and 262); the pH was unknown, but was probably basic. In a repeat experiment with the unadsorbed material from the cation exchange column, adjustment to pH 7 gave a response (No. 272), but adjustment to pH 2 gave no response (No. 277). There was the possibility that loss of attraction was related to pH.

In another series of experiments designed to check the stage at which loss occurred and the effect of strong and weak exchangers, bigeye flesh after extraction with alcohol was again treated with lead acetate, filtered, treated with sodium chloride (fig. 13), and divided into aliquots such that each test contained the treated extract from 400 g. of flesh. One portion gave an excellent response (No. 273) even after heating (see above). A second portion was passed through a weakly acidic (Duolite C-3) cation exchanger and the filtrate was tested, with a weak positive response (No. 278). A third portion was passed through a strongly acidic (Amberlite IR-120) cation exchanger and the filtrate was tested, with negative results (No. 274). Fourth and fifth portions were treated as above, but the filtrate from the strong cation exchanger was passed through a strongly basic (Amberlite IRA-400) anion exchanger (No. 275) and through a weakly basic (Duolite A-3) anion exchanger (No. 276), and the eluates tested, in each case with negative results. The only noticeable reaction with materials fractionated with ion exchangers was with the filtrate from the weakly acidic column. Again, the negative results may have been due to the unresponsive condition of the fish (although they reacted well in No. 273) or to the rather extreme changes in pH which occurred in passage through the ion exchangers --from about 7 to 3 with the cation exchangers and from about 3 to 12 with the anion exchangers.

Another series, designed to check the stage at which loss of the attractive substance occurred and the effect of pH, is depicted in figure 14. Yellowfin flesh was extracted with alcohol, the filtrate was acidified with hydrochloric acid to precipitate the lead as lead chloride (rather than as sodium chloride, as before), and filtered. One portion of the filtrate gave a good reaction when tested (No. 280). A second portion of the filtrate was evaporated to dryness over a water bath and dissolved in absolute ethyl alcohol,

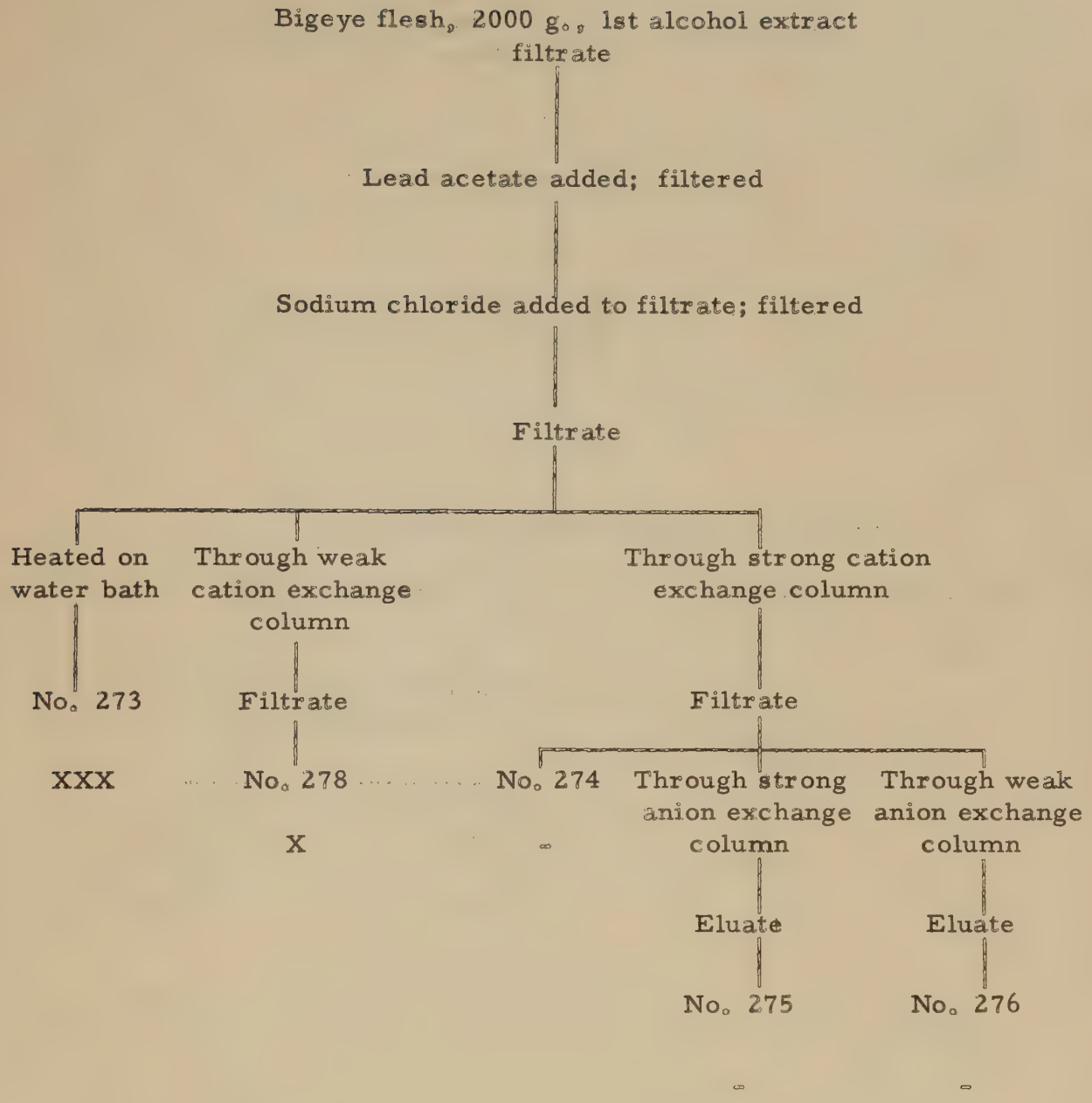


Figure 13.--Fractionation of a first alcohol extract of bigeye flesh: procedure and results in experiments Nos. 273 to 276, and 278.

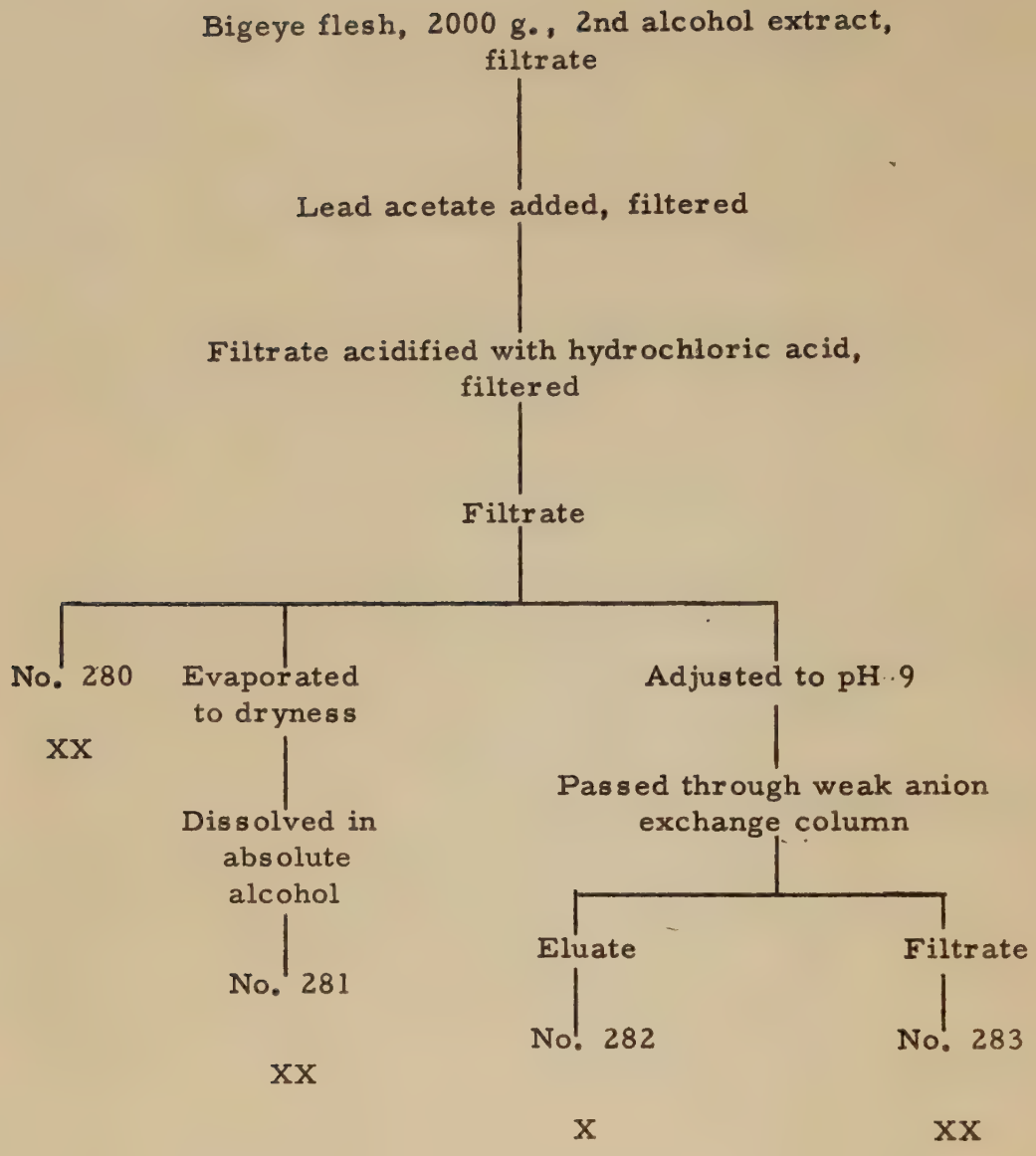


Figure 14.--Fractionation of a second alcohol extract of bigeye flesh: procedure and results in experiments Nos. 280 to 283.

again giving a good response when tested (No. 281). A third portion was adjusted to pH 9 with ammonium hydroxide, passed through a weakly basic (Duolite A-3) anion exchange column, and the adsorbed material was eluted with weak ammonium hydroxide and tested, giving a positive but weak response (No. 282). The unadsorbed filtrate, however, gave a stronger response (No. 283). Apparently there was only partial separation of the active substance on the anion exchanger. The fact that the results were generally positive, as compared with the negative results of the previous series, is surprising as they were based on a second and presumably weaker alcohol extraction of the same material as was used previously. The main differences were the precipitation of lead with hydrochloric acid rather than sodium chloride and the elimination of the cation exchanger, neither of which should have led to the differences. They may have been caused by either aberrant fish behavior or slight differences in technique.

In a third alcohol extraction of the same tuna flesh as used previously (fig. 15), the filtrate was treated with lead acetate, filtered, the filtrate was treated with hydrochloric acid, filtered, the filtrate was adjusted to pH 7, passed through a strong (Amberlite IR-120) cation exchanger, and the filtrate of pH 2 was tested with negative results (No. 285). When the filtrate from the cation exchanger was adjusted to pH 8 with ammonium hydroxide, there was a positive response (No. 286). When the material adsorbed on the cation exchanger was eluted with weak acetic acid and tested, it gave an even stronger response (No. 287). Again, the results are puzzling. On prior tests, the attractant had not been adsorbed (to any great extent, at least) on the cation exchanger. For the material that was not adsorbed, the attractive qualities seemed to be lost at a low pH.

At this time it was suspected that the variability and inconsistency of the results might have some relationship to the pH of the materials and the length of time they were subject to extremes of pH. In passing through cation and anion exchangers, and in other treatments which were used, pH's ranging from 2 to 12 were commonly induced for varying periods of time.

Several preliminary experiments with skipjack viscera preparations (Nos. 288, 289, 290; Nos. 295, 296, 297) and bigeye flesh preparations (Nos. 292, 293, 294) in which extracts exposed to pH's of 2, 7, and 10 were compared, gave inconsistent results but suggested that the response might be less at the two extremes; the mean (observational) scores for the three sets of experiments listed above were 1.0, 1.8, and 1.5 for pH's of 2, 7, and 10, respectively.

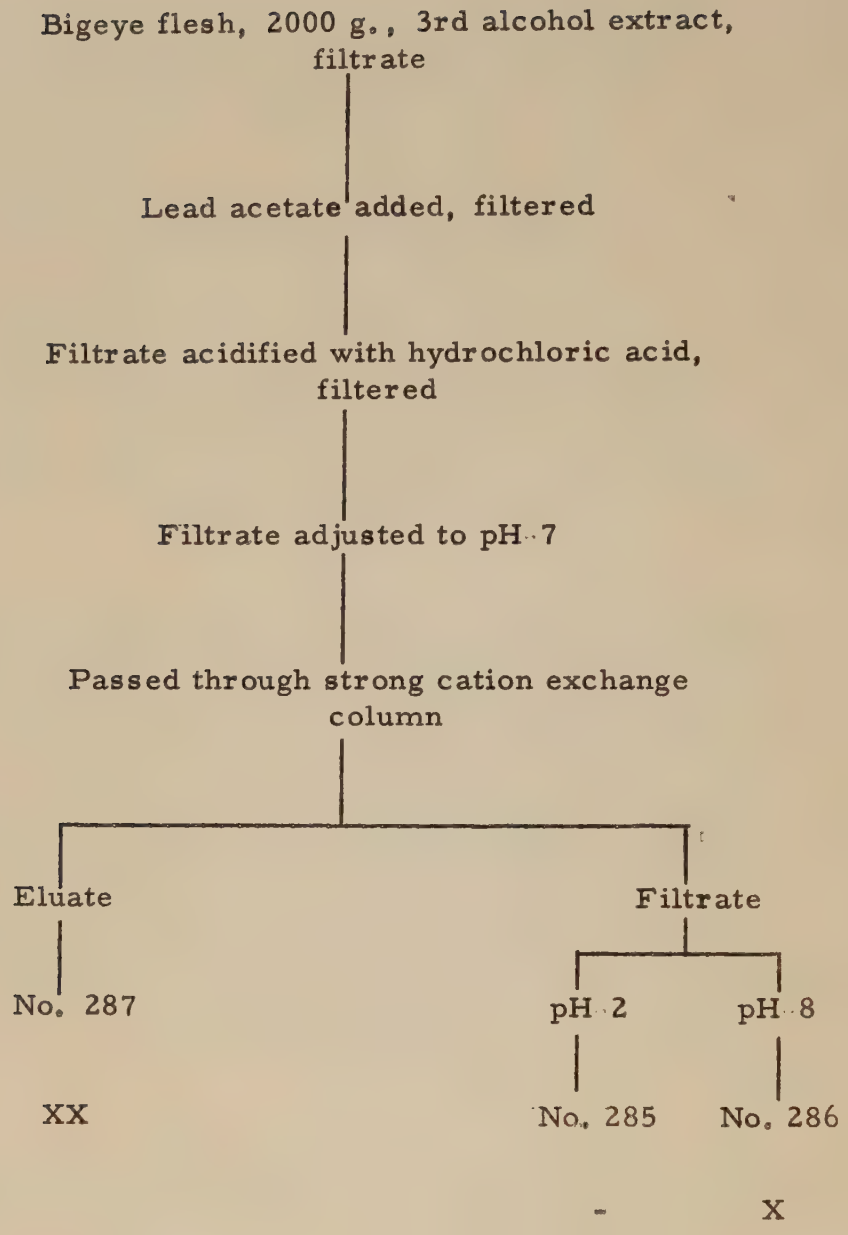


Figure 15.--Fractionation of a third alcohol extract of bigeye flesh: procedure and results in experiments Nos. 285 to 287.

A series of experiments was designed to test the hypothesis that the phosphate ion, assumed to be an integral part of the attractant, was made available at a high or low pH through hydrolysis, and that this was then precipitated (and lost) as magnesium ammonium phosphate by reaction with the seawater when the test substance was introduced. Bigeye flesh was extracted with alcohol and the filtrate was adjusted to pH 3 with hydrochloric acid to make the phosphate available for later removal. The filtrate was divided into six parts, each treated differently as shown in figure 16. Since there was a strong response to that part subjected to the most drastic method of removing the phosphate (No. 303), namely, adjusting to pH 1, boiling 15 minutes, adding 0.2 g. magnesium chloride, adjusting to pH 9, standing overnight, and then filtering and testing, the hypothesis that attraction is associated with labile or easily removed phosphate was discarded as untenable.

Finally, a series of experiments was designed to investigate the variability of the data (with only two tunny left in the pond), the effects of pH, and the effects of time lag between preparation of the material and testing. This series, conducted over a 3-week period in April 1952 (No. 313 et seq.), has already been discussed from the point of view of variability of the data. As may be calculated from table 3 (using eight complete replicates) the mean (observational) scores were 1.8, 2.1, and 1.3 at pH's of 2, 7, and 10, respectively. Although the results are suggestive of decreased response at the two extremes of pH, the differences when tested with either the observational or the quantitative data are not significant statistically. Moreover, the relative superiority of the acid and base are reversed as compared with the preliminary results given in a preceding series with skipjack viscera extract.

The preparations were made on a Saturday and were tested in two replicates on the following Tuesday or Thursday by one observer, and again in two more replicates on the following Saturday by a second observer. Thus the material tested by one observer was prepared for a shorter period of time than that tested by the second observer. The data, although incomplete for reasons discussed previously, yield 12 paired comparisons which may be segregated as follows according to time duration and pH:

	Shorter time (Observer 1)	Longer time (Observer 2)	Number of comparisons
pH 2	2.1	1.7	5
pH 7	1.4	2.1	4
pH 10	1.3	1.2	3

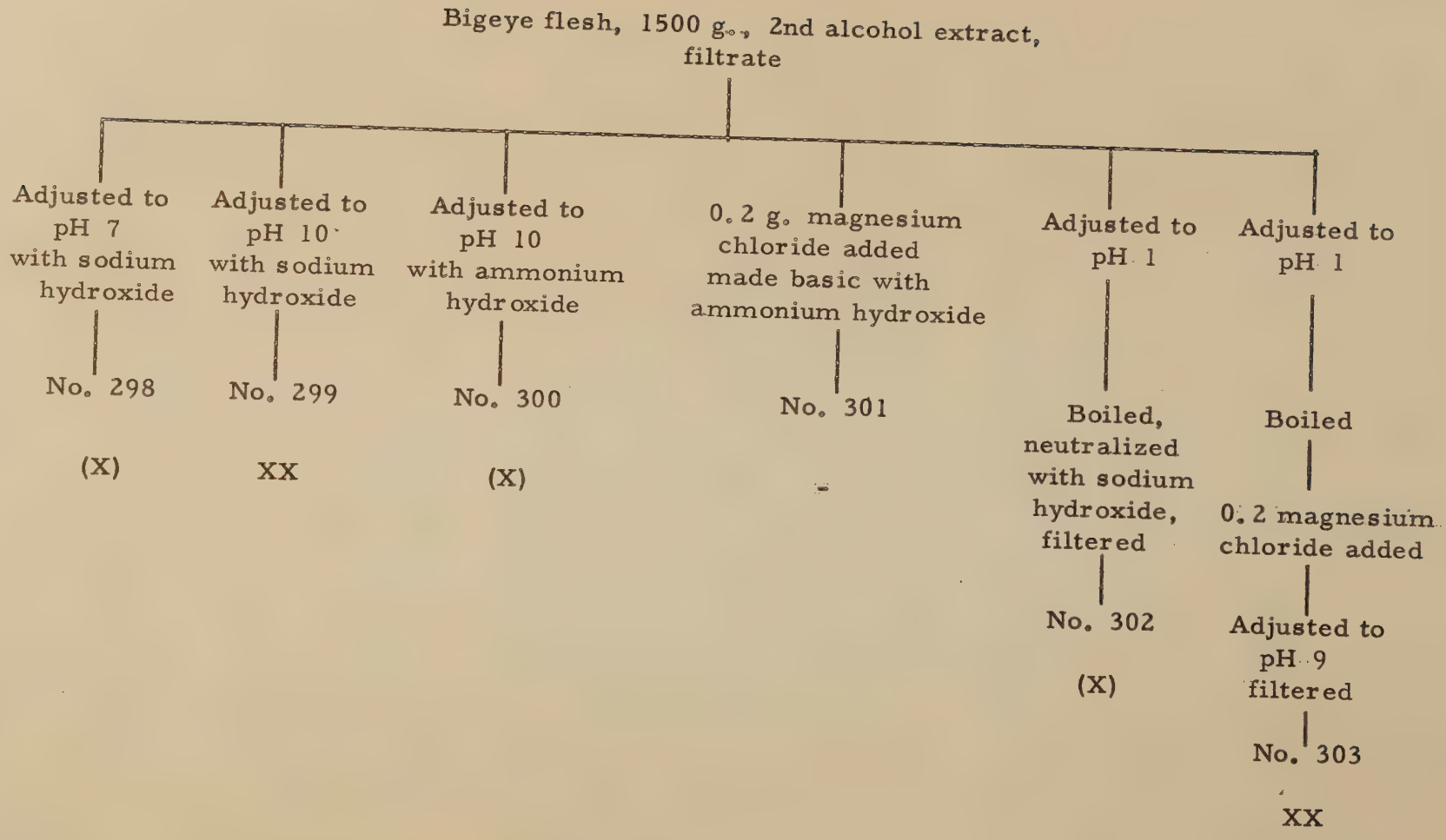


Figure 16.--Fractionation of a second alcohol extract of bigeye flesh: procedure and results in experiments Nos. 298 to 303.

The data suggest that at pH 2 the shorter the period between preparation and testing the greater the response. Again, however, the difference is not statistically significant. Moreover, an even greater difference in the opposite direction is present at the neutral pH 7. Also, of course, any differences due to time may be confounded with possible differences between observers.

The experiment provided no proof that differences in either pH or in time lag at various pH's caused differences in reponse. Rather, it emphasized the great variability of the response --a factor which doubtless contributed mostly to the erratic results encountered after the middle of December 1952. In view of this, further attempts at isolation and identification of the attractant were abandoned.

SUMMARY AND DISCUSSION

General

The present investigation, conducted from June 1, 1952 to May 31, 1953, is a study of the response of captive tunny to extracts of fish flesh, viscera, etc. and to certain chemicals in solution or suspension.

The tunas, caught by trolling, were transported to shore and established in a small concrete tank and a large pond at the Hawaii Marine Laboratory, Coconut Island, Oahu. For the most part, they were fed strips of tuna flesh 3 or 4 times a week and, on this diet, more than tripled their weight during the course of the year. The two tunny established in the tank during the summer of 1952 survived until the following summer. The population of 13 tunny, established in the pond, was decimated in two periods of mortality during the fall and winter; two fish survived to the following summer.

Test substances were introduced into the tank or pond through a continuous stream of water supplied by an electric pump. When and where possible, precautions were taken to avoid distraction of the fish by extraneous audio or visual stimuli.

When certain test substances such as clear aqueous or alcohol extracts of tuna flesh were introduced into the tank or pond, a response was obtained. This took the form of a feeding reaction and included one or all of the following components: speeding or acceleration of the swimming rate, a return to the area of distribution of the substance, surfacing, fanning-out and eventually a breakdown of school formation, circling, splashing, and biting at incidental objects on the surface of the water.

Although a positive response was obvious to the observer, it was extremely difficult to measure quantitatively because of its complexity and also at times because of erratic behaviour of the fish. Two quantitative measures were used, namely (1) the time out of 2- (tank) or 3- (pond) minute periods spent by the fish in an "attraction" area in which the test substance was dispersed, and (2) the number of "passes" (movements of each fish in one of two directions) in the attraction area. These measurements were usually made during five control periods (before introducing a substance) and during five test periods (after introducing a substance). A roughly quantitative (observational) measure of the strength of a response in one of five categories (- to XXXX) was also recorded for each experiment. With either measure, the response to the same test substance was extremely variable in both manifestation and strength, producing apparently inconsistent results in many of the replicated experiments.

It is postulated that in tuna flesh there is present a substance (or substances), herein called the attractant, which is perceived by the tunny through its sense of smell or taste and which promotes the urge to feed.

The attractant was present in the flesh, viscera, and blood of several species of tuna and also in the flesh and blood of certain white-fleshed fish. It was not present in beef blood. It was found in tuna cannery byproducts such as viscera preparations, stickwater, and fish meal.

Some 40 chemical compounds were tested as time permitted, including certain amino acids, vitamins, aromatics, proteins, etc. In some, notably certain amino acids, vitamins, and aromatics, there appeared to be a sensing of the dissolved or suspended compound but the results could not be duplicated. In no case did the response include all of the components of a typical reaction to a tuna flesh extract.

Nature of the Attractive Substance

Much of the work was devoted to attempts at purification, fractionation, and identification of the unknown attractant. Its properties are summarized in the paragraph which follows:

The attractive substance is soluble in water and alcohol but not in petroleum ether or acetone. It is not destroyed by cold or by heat (although it may be partly precipitated on boiling an extract which contains it). It is not precipitated by sodium chloride, tannic acid, lead acetate, phosphotungstic acid, or other substances which

remove proteins and purines. It will pass through a dialyzing membrane (in part, at least). It is not adsorbed (to any large extent) on activated carbon. It may be loosely adsorbed on columns of activated alumina or ion exchange resins. Of the latter, it appears to be held to a greater extent on an anion exchanger than on a cation exchanger, although the results are not consistent. It is affected slightly, if at all, by radical changes in hydrogen ion concentration. In its purest form yet attained, chemical tests showed the presence of phosphorus (but not sulphur), the amide link, and the benzene ring.

It is not yet possible to identify the attractive substance(s) from its chemical properties. It does not appear to be an amino acid, a fatty acid or lipoid, a purine, or a protein. In many of its properties it resembles Vitamin B_{12}, but this substance, while sensed by the fish, did not promote a typical positive response when tested.

Preservation of the Attractant

As sea testing of the extracts was contemplated, considerable interest centered on methods of preservation. Aqueous extracts of flesh and viscera could be kept for 2 or 3 weeks at temperatures at or just above the freezing point without excessive putrefaction and loss of activity. Aqueous extracts could be preserved indefinitely in a 2-percent solution of sulphur dioxide gas, sulphuric acid, phosphoric acid, or sodium bisulphite; apparently these chemical preservatives were not repulsive to the fish. Alcohol extracts could be kept indefinitely. Both water and alcohol extracts could be boiled to dryness, producing a dark brown, gummy residue which could be kept without deterioration for long periods of time, particularly if suspended on sodium chloride crystals; when the gummy substance was re-dissolved in water it was still attractive to the fish.

Possibility of Conditioning of the Fish

The question arises as to what extent the typical response to extracts of tuna flesh and viscera is promoted by a conditioning of the tunny to life in captivity and, particularly, to the type of food fed and the method of feeding.

The life of the tuna in the tank and pond differs greatly from that in the sea. The fishes' movements are limited by the walls and they fall into a pattern which is often precise and regular--much different from that of the open sea. Not only are their movements limited horizontally but they are also limited vertically by the shallowness of the water.

To survive in the pond or tank the tuna must become conditioned to feeding on inert, dead rather than motile, living food. After becoming conditioned to feeding on food such as strips of aku flesh, the fish appeared to have little interest in live food such as small baitfish (Stolephorus and Pranesus), which at times were present in the pond in fair abundance. On rare occasions they were observed to pursue the baitfish, which usually schooled near the seaward gates of the pond, but they would give up when the baitfish receded to a position close to the gates. They were not observed to actually capture any, and usually they ignored them. On the other hand, the tunas would almost invariably circle and snap at objects other than food which were thrown or fell accidently on the water surface (leaves, pieces of cellophane, etc.). This response was probably conditioned by the method of feeding. When a person appeared at the edge of the pond with a bucket of food, the fish would often see him and gather nearby, waiting for the food to be thrown to them. When it was thrown, they would follow its path and take it immediately it landed on the surface.

It is possible that they were conditioned to being fed at regular intervals of time (usually 4 p.m. every second day), although they would respond to the presence of both persons and food at any time except immediately after being fed.

As most of the extracts which were used were not visible to the fish, there is no doubt that the response was through the fish's sense of smell or taste rather than through its vision. However, there is the question as to whether the fish responded to skipjack flesh extracts because they were being fed pieces of skipjack, or closely-related tunas, as a regular diet. There is evidence that the response was not conditioned by the type of food which was fed.

Before and during the early experiments in the tank (to July 18, 1952) the one established tunny was fed a non-tuna diet (squid and baitfish), yet it still responded to extracts of skipjack flesh (Nos. 1, 8, 10) which it had not tasted while in captivity. Following April 25, 1953, the two tunny in the pond were fed exclusively on squid, yet almost 1 month later, May 19, 1953, they still responded to extracts prepared from skipjack viscera and yellowfin flesh (Nos. 350 to 356).

The fact that positive responses were obtained to extracts of fish other than tuna--aholehole (Nos. 49, 88, 105), jack (No. 96), and barracuda (No. 98)--while the tunny were still being fed skipjack is also evidence that the response was not conditioned by the species of fish which was being used as food. This, of course, is also

illustrated by responses to extracts of tunny and yellowfin while the fish were being fed on skipjack, and responses to extracts of skipjack while the fish were being fed yellowfin or bigeye tuna.

It may be concluded that the response of the tunny in captivity is not directly conditioned to the species of food which was fed. However, there still remains the possibility that the tunny formed a mental association between feeding and the smell or taste of the dead food, which was cut up or otherwise macerated and exuded juices of similar composition to the extracts. An association such as this would not necessarily be present in the case of wild fish feeding on whole living organisms at sea. This, or some other subtile type of conditioning to life in the pond, may have contributed to the response.

The part played by conditioning can be ascertained only by testing the extracts on schools of fish at sea. Certain materials for sea testing have already been prepared and pond-tested (Nos. 264, 279, 291, 304 to 312, 350 to 356). The results of sea tests of these and other preparations will be discussed in a later report. For the present it may be stated that the preliminary sea tests were largely negative.

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PART II

RESPONSE OF TUNA TO VISUAL AND VISUAL-CHEMICAL STIMULI

BY

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During September 1952 a few experiments were conducted on a population of 12 little tunny1/ (Euthynnus yaito) established in Pond No. 5 at Coconut Island, Oahu, to determine (1) whether visual lures would promote a tropistic response, (2) whether the response was heightened when combined with chemical stimuli, i. e., an extract of tuna flesh, and (3) whether the response varied with lures of different colors. It was hoped that the results would be of interest and value in devising lures which, either alone or in combination with extracts, would assist in attracting tuna to the stern of fishing vessels at sea.

METHODS

The lures consisted of 2-inch sections of 1/2-inch rubber tubing of different colors which were suspended from a cross bar attached to the tip of a 25-foot bamboo pole, pivoted near the point of balance (fig. 17). By means of a cord, a counterweight, and a system of pulleys, two lures, one suspended from each end of the cross bar, could be momentarily dipped into the water. When an experiment was in progress the lures were lowered about once every 2 seconds.

The lure array was fastened near the base of a 20-foot observation tower and could be manipulated from the platform on top. Observations of the fish were made when they entered the 39 x 75-foot attraction area (the same area that was used in chemical stimulation studies) marked off by two pieces of cord stretching across the pond.

Note: Contribution No. 48 of the Hawaii Marine Laboratory, University of Hawaii, Honolulu, T. H.

1/ The activity of one "sick" tunny was not recorded.

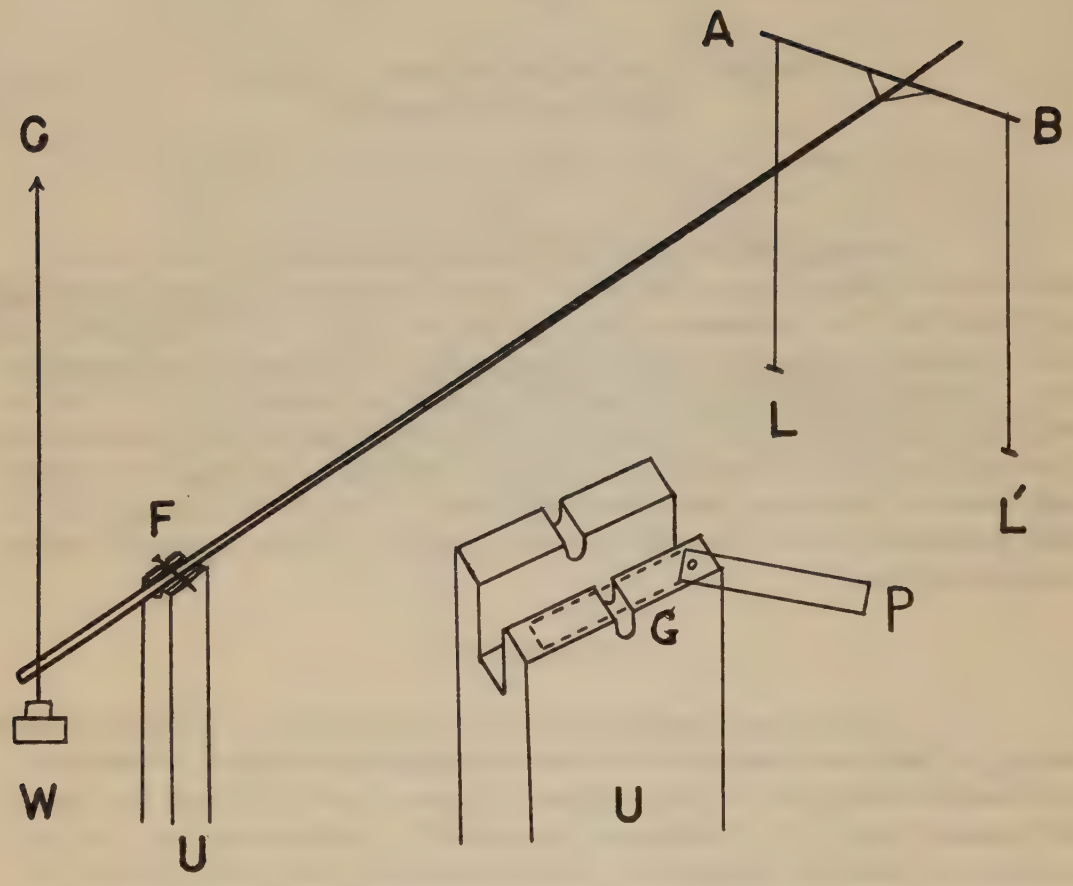

Fig. 17.--Diagram of mechanical device for lowering lures into water: AB - cross-piece; LL' - lures; U - supporting post; F - fulcrum; C - notch; P - plate to cover notch; W - weight; C - cord.

All experiments were conducted while the pump at the seaward or western end of the pond was operating. This projected a strong current of water into the first 1/10th of the pond and a weaker current into the second 1/10th, the attraction area. In some experiments, the lures only were used; in others, stock extracts of tuna flesh were introduced through the pump flow at the same time as the lures were presented. A detailed description of the pond, pump, stock extract, normal behavior of the fish, etc., is given in Part I of this report by Tester, van Weel, and Naughton, and the general arrangement for testing is shown in figure 4.

In a typical experiment the pump was switched on and the observers on top of the tower waited until the fish were behaving in a normal fashion, i. e., cruising slowly back and forth along the length of the pond in one or two schools. The activity of the fish was observed and recorded during a 15-minute control period (the data could be divided into 3-minute intervals). The lures were then dipped momentarily into the water once every 2 seconds and the activity of the fish was observed and recorded for a 15-minute experimental period. The colored lures were then reversed in position (switching left to right and right to left) and the observations were repeated. After two or more 15-minute experiments with lures, stock extract was added, the lures were dipped into the water, and observations of activity were made and recorded for another experimental period. The lures would then be switched, or changed, and the experiment repeated. In some of the early tests (9/3 and 9/5) the control and/or experimental periods were of shorter duration (9 or 12 minutes).

The behavior of the tunny, particularly during experimental conditions, was exceedingly complex. It was difficult to measure the activity with the usual devices--electrically controlled time clock, stop watch, and mechanical counter--and to record the data with pencil and paper, even when two observers were working together on top of the tower. A system of recording the behavior was worked out which consisted eventually of (1) the use of a tape recorder to give a running verbal description of the activity of the fish, and (2) the transcription of this information on the smoked paper of a kymograph drum by operating a series of levers to which styli were attached. The transcribing apparatus is illustrated in figure 18. It consists of a kymograph (F), a Franz kymograph timer (C) with two recording points, a lever (B) and stylus attached by a cord to the axle of a telephone dial (D) so that the length of the stroke on the drum is proportional to the number dialed, and a lever (A) and stylus attached by a rigid rod to a toggle key (E) which can be flipped up or down from a neutral position to make an up or down stroke on the smoked paper. The time is recorded by C, the number of fish in

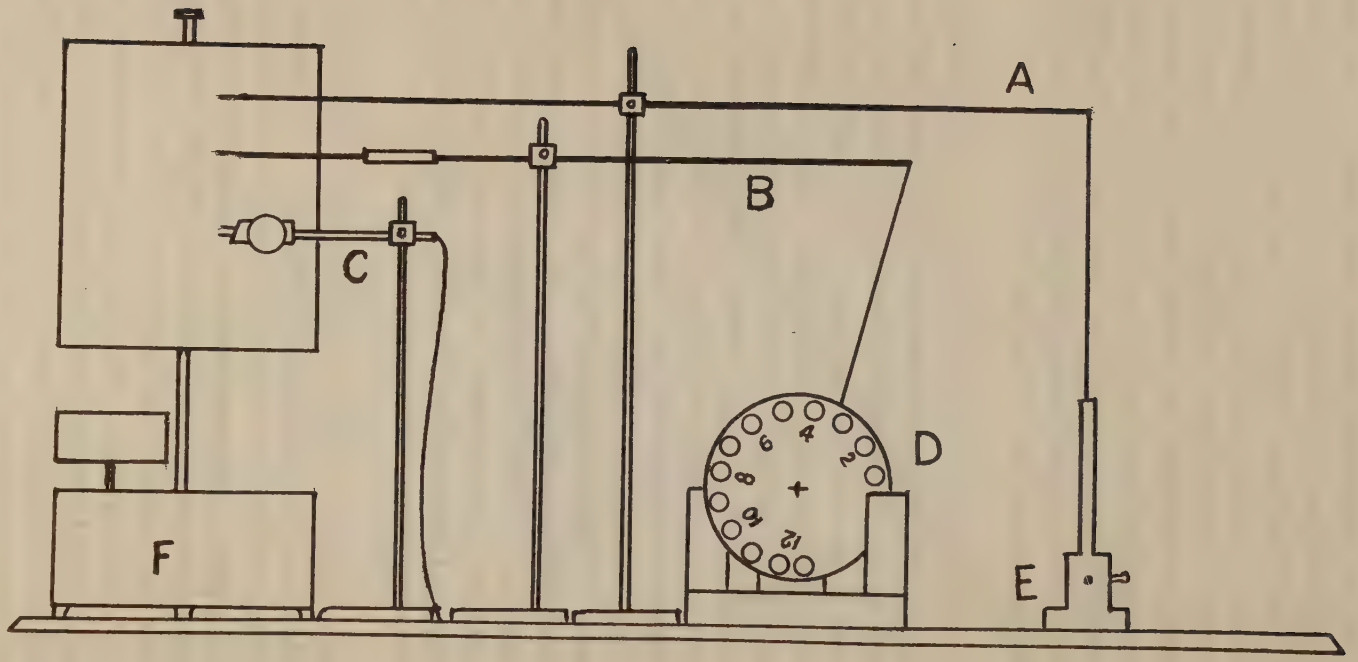

Fig. 18.--Diagram of transcribing-recording device: A - lever operated by toggle switch (E) for recording "passes"; B - lever operated by dial (D) for recording number of fish in area; C - timer; F - kymograph.

the area is recorded by B, and the number of passes at one (up) or the other (down) of the two lures is recorded by A. Typical recordings are shown in figure 19.

In addition to the above, the speed of the tunny was determined in several trials under control and experimental conditions by timing the leader of the school as it passed through the attraction area, using a stop watch.

RESPONSE UNDER CONTROL AND EXPERIMENTAL CONDITIONS

The pattern of activity of the fish was observed under control and experimental conditions and is discussed below. The activity was measured quantitatively in several different ways, each of which will be presented and discussed in the sections to follow.

Pattern of Response

During control periods the tunny, usually in one school or in two schools of unequal size, swam slowly back and forth along most of the length of the pond, looping in deeper water near the ends, or often pausing to "play" in shallower water at the ends before looping.

A kymograph record of observations during a 15-minute control period in one experiment is shown in figure 19A. It is discussed in detail to illustrate the interpretation of the kymogram. The first histogram shows that 22 seconds after the start, a school of 12 tunny entered the field from the east. After spending 24 seconds in the field, they left by the same side as they came in, and swam to the eastern end of the pond. After 165 seconds the school returned and swam through the field in 10 seconds, as shown by the second histogram. The school looped in the western 1/10th of the pond during a 9-second interval and re-entered the area from the western side. As shown by the third histogram, they sped through the field in 8 seconds. After 147 seconds the schools re-entered the area from the east, stretched out in linear formation, with the leader entering the field about 6 seconds ahead of the 12th tunny (fourth histogram). After leaving the field, the school spent 84 seconds in the western 1/10th of the pond, during which time they circled in the shallow southwestern corner near the pump. After passing through the field for a fifth time, the tunny stayed in the eastern part of the pond for a long period--about 4 minutes--before returning to pass through the area (sixth histogram). When they entered the area, 2 turned back, but 10 passed through to spend 60 seconds in the

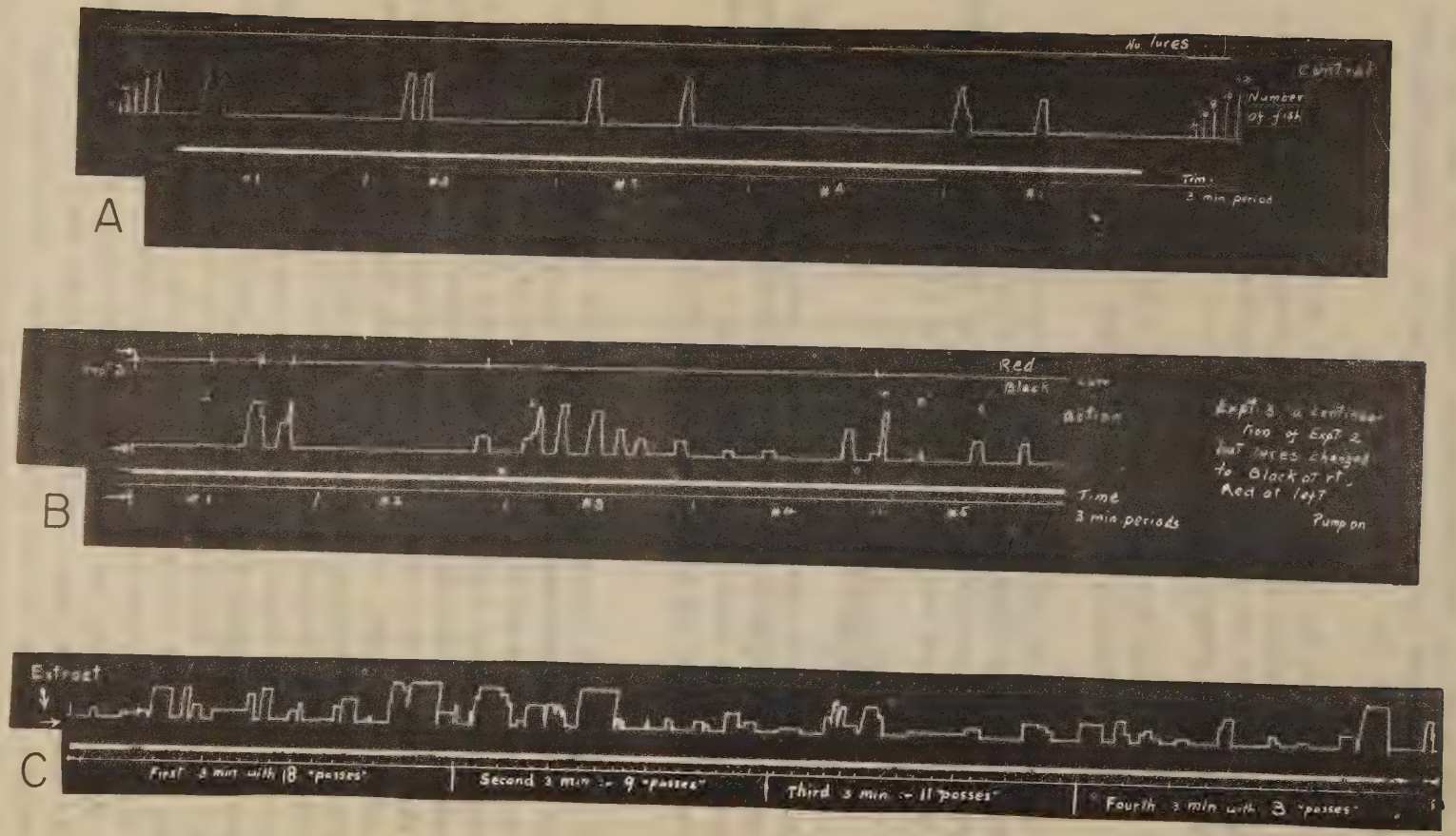

Fig. 19.--Typical kymograms: A - under control conditions; B - when stimulated by lures; C - when stimulated by lures plus extract.

western 1/10th of the pond. On turning back they sped eastward through the field in 12 seconds (seventh histogram). The two tunny which separated from the school earlier circled slowly outside the eastern border of the field, and 15 seconds after the major school left these two fish re-entered the area and swam inside for 18 seconds (eighth histogram).

When a pair of colored lures was lowered repeatedly into the water inside the attraction area, one or more tunny might be moving westerly towards the area and near enough to see the moving objects. In this case they would usually swim toward the lures and try to take them. It often happened that when one or two tunny started to bite, the others near them would become active and circle back to the lures. On the other had, if the tunny were at the far end of the pond, the lures might be lowered repeatedly for a long time without any response from the fish until they approached the attraction area. When taking after the lures, the speed of movement was not noticeably increased compared with that during the control period.

Figure 19B shows the reaction of the tunny in an experiment in which two lures, one red and the other black, were presented. They entered the field 17 times in groups of different sizes. In the third 3-minute interval, attraction and excitement is indicated by the close spacing of the histograms. The symbols "C" and "O" in the figure indicate looping and circling, respectively, inside the area. From the strokes indicating the response to the lures (upper line), it will be seen that they attempted to "take" the red lure 8 times and the black lure 6 times.

When a stock extract of tuna flesh was introduced through the stream of water at the western end of the pond the tunny would become greatly excited as soon as they sensed it. Usually the extract was propelled into the attraction area about 1 minute after its introduction. The fish would dash about with increased speed, biting at floating objects such as leaves and sticks, as well as running for the lures and fighting among themselves. There was a clearly visible feeding reaction. But after 10 to 15 minutes the response would gradually disappear and they would assume a pattern similar to that seen when lures alone were used.

In figure 19C a kymograph record of the response to lures and extract is seen. The number of passes at the lures is indicated by numbers rather than by strokes. The great excitement when extract was added is evident from the increased frequency of the histograms, together with their varied shape. The fish took the

lures 18 times in the first 3-minute interval, 9 times in the second, 11 times in the third, and 3 times in the fourth.

These and other kymograph records are analyzed more extensively in the sections which follow.

School Entrances

The activity under various conditions of stimulation may be measured by the number of schools (of varying size) entering the attraction area during a 15-minute period. The results of the experiments are summarized in table 4. In this, and other tables to follow, the data for September 5 and 9 have been adjusted to a 15-minute period. The results for similar experiments conducted on the same day have been averaged.

It will be seen that on each day schools entered the attraction area more frequently (as compared with control conditions) when a pair of colored lures was dipped into the water, and still more frequently when lures were used together with extract. The grand mean number of entrances was 6.7 for controls, 14.6 for lures, and 21.6 for lures plus extract. Obviously the differences between the means are statistically significant.

Table 4.--Summary of data on the number of schools entering the attraction area under various conditions of stimulation

Condition	Date						
	9/3	9/5	9/8	9/10	9/12	9/17	Mean
Control	10	5	9	3	7	6	6.7
Lures	19.25	13	-	12.5	16	12	14.6
Lures plus extract	38.25	21.25	22.33	14.00	16.75	17	21.6

Percentage of Time in Area

The percentage or relative time spent by the fish in the attraction area is summarized in table 5 for the various conditions of stimulation. On the average the time spent in the area was 12.0

percent during control conditions, 24.5 percent when two lures were used, and 29.5 percent when the lures plus extract were used. A rough test (using "t") of significance of the difference of the paired observations (neglecting the varying intrinsic accuracy of the mean determinations for each day's experiments) indicates a significant difference between control and lures (mean difference, 13.8 percent; $P < 0.01$), but not between lures and lures plus extract (mean difference, 4.0; $P = 0.2$).

Table 5.--Summary of data on percentage time spent in the attraction area under various conditions of stimulation

Condition	Date							
	9/3	9/5	9/8	9/10	9/12	9/15	9/17	Mean
Control	9.6	6.9	20.0	4.0	14.0	15.6	14.2	12.0
Lures	27.0	17.0	-	23.5	32.7	23.8	23.1	24.5
Lures plus extract	35.0	27.8	33.7	21.3	32.7	-	26.4	29.5

Fish-seconds in Area

The summation of the product of the number of fish and time spent in the area is probably a better measure of activity than the two measures discussed above. This is equivalent to summing the areas under the histograms in the kymograph records, and may be expressed in fish-seconds per 15-minute period. The results are given for individual experiments in table 6.

In comparison with the controls, it will be observed that the number of fish-seconds is generally greater when the lures are used, and slightly greater still when the lures are combined with extract. The grand means for control, lures, and lures plus extract are respectively 114.3, 352.8, and 364.8 fish-seconds. In experiments with lures (alone) which were repeated in succession on the same day, the measure tends to fluctuate rather widely, showing neither a consistent increase nor decrease in response. In experiments with lures plus extract, the measure tends to decrease with repeated stimulation, indicating a dulling of the response. There are five comparisons of control, lures, and lures plus extract involving

Table 6.--Number of fish-seconds spent in the attraction area under various conditions of stimulation

Date	Condition		
	Control	Lures	Lures plus extract
9/3	47	(1) 253 (2) 640 (3) 101	(1) 522 (2) 107
9/5	100	(1) 683	(1) 371
9/8	120	-	(1) 657 (2) 310 (3) 345
9/10	34	(1) 646 (2) 165	(1) 380 (2) 167
9/12	135	(1) 453	(1) 504 (2) 319 (3) 292 (4) 289
9/15	124	(1) 207 (2) 168 (3) 115	-
9/17	240	(1) 80 (2) 370	(1) 635 (2) 209

initial experiments, indicated as "(1)" in table 6. These yield the following comparable means: 111.2, 423.0, and 482.4 fish-seconds, respectively. Obviously, the means for both lures alone and for lures plus extract are both significantly greater than that for controls. Although the mean for lures plus extract is greater than that for lures, the statistical significance of the difference cannot be established with the present data.

Speed of Swimming

Another measure of activity is speed of swimming. This was determined by timing the leader of the school as it passed between the two lines marking the boundary of the attraction area.

Sources of error include the effect of parallax in determining when the fish is exactly below the lines, deviation from a path exactly perpendicular to the lines, and deviation from a horizontal plane. However, variation induced by these sources of error is small compared with the variability from trial to trial. The results of observations under control and experimental conditions are summarized in table 7.

Table 7.--Summary of data on speed of swimming under various experimental conditions

Condition	Number of observations	Mean time and standard error	Mean speed (feet/second)
Control	12	18.42 ± 1.60	2.12
Lures	12	15.08 ± 0.90	2.59
Lures plus extract	20	10.15 ± 0.63	3.84

The mean speed of swimming increased beyond that of the controls when the tunny were stimulated by lures, and it increased still more when they were under the combined stimulus of lures and extract. The mean difference between control and lures is not significant statistically ($P = 0.08$). However, that between lures and lures plus extract, and also that between control and lures plus extract are both highly significant ($P < 0.01$ in each case). The increased rate of swimming of the tunny when stimulated by the extract was obvious to the observer. The actual mean swimming rate under these conditions (3.84 feet per second) is equivalent to 2.6 miles per hour. Of course, bursts of speed several times this magnitude were frequently observed.

Feeding Activity

Feeding activity may be measured by the number of passes (bitings or attempted bitings) at the lures both when used alone and when used in conjunction with extract.

Table 8 shows the mean number of passes at the lures per 15-minute period on 5 successive days. The grand means for lures and for lures plus extract are respectively 14 and 40 passes

per 15-minute period. The increased feeding response under the additional stimulation of extract is apparent without further statistical analysis. The data are not suitable for isolating and testing the component of variance associated with daily variation in response.

Table 8.--Mean number of passes at lures under two experimental conditions

Date	Condition	
	Lures	Lures plus extract
9/3	26.7	51
9/5	13	20
9/10	10.5	40
9/12	6	29
9/17	14	20

RESPONSE TO LURES OF DIFFERENT COLORS

Each lure of a pair differed from its mate in color. The following combinations were tried: white-red; white-black; white-silver; red-black; and silver-black. As already explained, the positions of the lures were interchanged between experiments. The number of passes at each lure per 15-minute period was determined when lures were used alone and when they were used in combination with extract. The results are summarized in table 9.

From the table it will be observed that the white lures received either the same or, more usually, a greater number of passes than the colored lures (either tested alone or with extract) when paired with red, black, or silver lures. Similarly, the red lure seemed superior to the black in number of passes. However, none of the ratios, either individually or grouped according to similar experiments, differ significantly from a 1:1 ratio when tested with Chi-square. From the pooled data for white versus all other colored lures with which it was paired there is evidence of the superiority of white. The ratio of pooled values for white vs. color is 155:122 which, when compared with the hypothetical 138.5:138.5,

yields an adjusted Chi-square of 3.7 with one degree of freedom ($P = 0.06$), which could be regarded as evidence for rejecting the hypothetical equality ratio. Although there is this evidence of a significantly greater number of passes at the white as compared with the colored lures, the superiority of the white lure is slight. Knowing that the tunny will make a pass at any inanimate object which appears on the surface, including not only lures but also leaves, sticks, pieces of paper, etc., the slight superiority of the white lure may perhaps be attributed to its greater visibility. There is no assurance that this superiority would be manifested in the sea, where conditions of sea water transparency and background color would be much different from those in the pond.

Table 9.--Number of passes at paired lures of different colors under two experimental conditions

Colors of paired lures	Conditions	
	Lures	Lures plus extract
White-red	(1) 5-5 (2) 10-8	(1) 31-27 (2) 7-4
White-black	(1) 8-5 (2) 4-4	(1) 10-9 (2) 12-7 (3) 7-2 (4) 21-19 (5) 6-5 (6) 15-10
White-silver	(1) 8-8	(1) 11-9
Red-black	(1) 3-3 (2) 9-9	(1) 23-20 (2) 16-13 (3) 9-6
Silver-black	(1) 11-8	(1) 12-12

SUMMARY

The activity of 12 little tunny in a large pond was observed during September 1952 under control conditions (no stimulation), when a pair of lures was dipped into the water once every 2 seconds, and when the lures were used together with stock extract of tuna flesh.

Observations were made in an "attraction area" which could be viewed from the top of a 20-foot tower. Activity was recorded verbally on a tape recorder, and later transcribed to a kymograph drum. The kymograms furnished quantitative data on activity.

As compared with control conditions, schools entered the area more frequently when stimulated with the lures, and still more frequently when stimulated with lures plus extract.

As compared with control conditions, the fish spent more time in the area when stimulated with lures, and still more time when stimulated with lures plus extract. However, the difference in relative time between the two experimental conditions was not statistically significant.

When activity was expressed as number of fish-seconds per unit time in the area, the results were similar to the foregoing. When lures and extract were used together, there was a dulling of the response with repeated testing.

The use of both lures and extract increased the rate of swimming from an average of 2.12 feet per second for controls to an average of 3.84 feet per second.

The fish made many more passes at the lures when stimulated with extract, showing a heightened feeding response.

Although, in general, the fish made more passes at white as compared with colored lures, the superiority of the white lures was slight. It may have been associated with greater visibility rather than color preference. There is no assurance that white lures would be superior to colored lures in the open sea.

APPENDIX

A summary of experiments on chemical stimulation conducted in tank (T) and pond (P) by van Weel (vW), Tester (T), or Naughton (N), over the period July 1, 1952 to May 19, 1953, with reaction classified in categories ranging from "-" to "XXXX".

Expt. No.	Date 1952	Substance and preparation	Tank or pond	Observer	Reaction	Remarks
1	7/1	Skipjack flesh, 150 g. extracted with 300 ml. water: centrifugate.	T	vW	XXXX	Tunny had not previously been fed skipjack flesh.
2	7/1	Same as No. 1: murky residue.	T	vW	-	Visual attraction only.
3	7/8	Ovaries, skipjack, 250 g. extracted with 500 ml. water, not centrifuged: whole extract.	T	vW	-	Cloudy material apparently repellent.
4	7/8	Testes, yellowfin, 400 g. extracted with 800 ml. water, not centrifuged: whole extract.	T	vW	-	Erratic behavior.
5	7/11	Skipjack flesh, 220 g. boiled 10' in 500 ml. water, filtrate.	T	vW	-	Erratic behavior.
6	7/11	Wahoo flesh, 170 g. extracted in 440 ml. water: centrifugate.	T	vW	-	No indication of attraction.
7	7/11	Same as No. 6: murky residue.	T	vW	-	"
8	7/14	Skipjack flesh, 225 g. extracted with 750 ml. water, not centrifuged: whole extract.	T	vW	X	Erratic behavior, but definite attraction.
9	7/14	Skipjack flesh, 282 g. boiled 10' with 600 ml. water: filtrate.	T	vW	-	

Expt. No.	Date 1952	Substance and preparation	Tank or pond	Observer	Reaction	Remarks
10	7/18	Skipjack flesh, 225 g. extracted with 500 ml. water: centrifugate.	T	vW	XX	Definite reaction.
11	7/18	Skipjack flesh, 282 g. boiled 10' with 600 ml. water: filtrate.	T	vW	-	
12	7/22	Skipjack flesh, 350 g. boiled 10' with 500 ml. water: filtrate	T	vW	-	Visual attraction to investigator at start.
13	7/22	Skipjack flesh, 240 g. extracted with 400 ml. water: centrifugate.	T	vW	X	Very slight attraction.
14	7/24	Yellowfin flesh, 50 g. extracted with 150 ml. water: centrifugate.	T	T	XX	Definite attraction.
15	7/24	Same as No. 14, murky residue.	T	T	XX	Definite attraction.
16	7/25	Skipjack flesh, 227 g. extracted with 500 ml. water: centrifugate.	T	vW	-	Fish had been fed 2 hours previously, slight attraction at beginning only.
17	7/28	Testes, yellowfin, 230 g. extracted with 500 ml. water, not centrifuged: whole extract.	T	vW	-	
18	7/28	Yellowfin flesh, 100 g. extracted with 200 ml. water: centrifugate treated with 3 x 50 ml. petrol ether.	T	vW	XX	

Expt. No.	Date 1952	Substance and preparation	Tank or pond	Observer	Reaction	Remarks
19	7/28	Ethyl alcohol, 60 ml.	T	T	-	Records lost.
20	7/28	Skipjack flesh, 203 g. extracted with 600 ml. water: centrifugate.	T	vW	XX	New fish present were distracting.
21	7/28	Skipjack flesh, 172 g. boiled 10' with 500 ml. water: filtrate.	T	vW	-	
22	7/28	Anethol, 6 ml.	T	T	(X)	Apparent attraction ?
23	7/29	Yellowfin blood, 100 ml. extracted with 500 ml. seawater, filtered through cheesecloth: filtrate.	T	T	XXX	
24	7/29	Yellowfin skin, 44 g. extracted with 300 ml. seawater: whole extract.	T	T	-	Possible visual repulsion.
25	7/29	Yellowfin liver, 82 g. extracted with 300 ml. seawater, filtered through cheesecloth: filtrate.	T	T	XX	
26	7/29	Yellowfin flesh, 100 g. extracted with 1000 ml. seawater, filtered through cheesecloth: filtrate.	T	T	X	
27	7/31	Tunny flesh, 100 g. extracted with 200 ml. dist. water, filtered through cheesecloth: filtrate.	T	T	XX	Attraction evident.

Expt. No.	Date 1952	Substance and preparation	Tank or pond	Observer	Reaction	Remarks
28	7/31	Tunny liver, 95 g. extracted with 200 ml. dist. water, filtered through cheesecloth: filtrate.	T	T	XXX	Attraction evident.
29	7/31	Tunny skin, 100 g. extracted with 200 ml. dist. water, filtered through cheesecloth: filtrate.	T	T	(X)	Slight attraction at first, then repulsion--visual?
30	7/31	Tunny blood, 150 cc. extracted with 300 ml. dist. water, filtered through cheesecloth: filtrate.	T	T	XX	Great excitement, but circling in attractive area only during 1st and 3rd periods.
31	8/1	dl-Phenylalanine, 2.5 g.	T	vW/T	-	
32	8/1	Arginine, 2.5 g.	T	vW/T	-	
33	8/1	l-Tyrosine, 2.5 g.	T	vW/T	-	
34	8/1	dl-Isoleucine, 2.5 g.	T	vW/T	-	
35	8/4	Gelatine, ca. 6 g.	T	T	-	
36	8/4	Eosin B, 0.6 g.	T	T	-	Slight repellent effect from this red dye.
37	8/4	Isoeugenol, 2.5 g.	T	T	-	

Expt. No.	Date 1952	Substance and preparation	Tank or pond	Observer	Reaction	Remarks
38	8/4	Methylene blue, 0.6 g.	T	T	-	Slight repellent effect from this blue dye.
39	8/5	Skipjack flesh, 252 g. extracted with 350 ml. water, centrifuged 7/31, centrifugate dialyzed: dialyzate.	T	vW/T	XX	Feeding reaction, but not clear-cut attraction of controls.
40	8/5	Same as No. 39: residue from dialysis.	T	vW/T	(X)	Slight attraction in first period.
41	8/5	dl-Valine, 2.5 g.	T	vW	-	
42	8/5	l-Taurine, 2.5 g.	T	vW	-	
43	8/7	Skipjack blood, 125 ml.	T	vW/T	XXX	
44	8/8	l-Leucine, 2.5 g.	T	vW/T	-	
45	8/8	dl-Norleucine, 2.5 g.	T	vW/T	-	
46	8/8	Tunny blood, 110 ml. dialyzed with 500 ml. dist. water for 48 hours: dialyzate.	T	T	(X)	No excitation, positive reaction doubtful.
47	8/8	Same as No. 46: residue dialysis.	T	T	-	No noticeable reaction fish not acting normally?
48	8/11	Skipjack skin, 48 g. extracted with 150 ml. dist. water, centrifuged 1-1/2 hours: centrifugate (still cloudy).	T	T	X	Some attraction, probably not visual as "cloud" not apparent in tank.

Expt. No.	Date 1952	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
49	8/11	Aholehole, 70 g. extracted with 210 ml. dist. water: centrifugate.	T	T	X	
50	8/11	Skipjack flesh, 1000 ml. stock extract 1/	T	T	XXX	
51	8/12	dl-Serine, 2.5 g.	T	vW/T	X	Fish seemed to "follow" the path of solution.
52	8/12	Skipjack flesh, 306 g. extracted with 500 ml. water, centrifuged 8/7, centrifugate dialyzed: residue from dialysis.	T	vW/T	XX	
53	8/12	Same as No. 52: dialyzate.	T	vW/T	X	Increased time spent in area of attraction but no great excitement.
54	8/12	Skipjack flesh, 5000 ml. stock extract.	P	T	XXXX	Great excitement, no record kept.
55	8/14	Beef blood, whole, 200 ml.	T	T	-	Visual attraction at first, then strong repulsion.
56	8/15	dl-Serine, 2.5 g.	T	T	(X)	Possibly some attraction but no excitement.

1/ "Stock extract" was prepared by grinding 9 lb. of skipjack flesh, adding 10 gal. water and extracting in refrigerator. The clear, reddish supernatant liquid was used in testing.

Expt. No.	Date 1952	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
57	8/15	Skipjack flesh, 1000 ml. stock extract dialyzed with 850 ml. dist. water 8/11, and with 1100 ml. dist. water 8/12, removed 8/14; residue from dialysis.	T	T	XXX	During controls, some of material leaked in causing premature attraction.
58	8/15	Same as No. 57: dialyzate.	T	T	X	Positive but weak attraction.
59	8/18	Skipjack flesh, 1000 ml. stock extract, boiled for 1/2 hour: centrifugate.	T	T	(X)	Very slight attraction, perhaps visual to froth on water.
60	8/18	Same as No. 59: residue.	T	T	-	
61	8/18	Beef blood plasma, 280 ml.	T	T	-	
62	8/18	Albumen, 100 g.	T	T	-	
63	8/18	Skipjack flesh, 1000 ml. stock extract.	T	T	-	Possibly a slight response? Fish not responsive, throwing doubt on all 8/18 experiments.
64	8/18	Skipjack flesh, 1000 ml. stock extract.	P	T	XX	5-minute timing intervals.
65	8/18	Skipjack flesh, 500 ml. stock extract.	P	T	XXX	" " "

Expt. No.	Date 1952	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
66	8/18	Skipjack flesh, 250 ml. stock extract.	P	T	XXX	Mistake in timing, first interval.
67	8/19	Skipjack flesh, 250 ml. stock extract.	P	T	XX	
68	8/19	Skipjack flesh, 1500 ml. stock extract, centrifugate ppted. with 10% HCl, centrifuged, neutralized: "non-protein fraction".	P	T	X	Slight delay in reaction.
69	8/19	Same as above, residue from second centrifuging, neutralized: "protein fraction".	P	T	XX	Good feeding reaction.
70	8/19	Skipjack blood, 50 ml.	P	T	XXX	Yellowfin also reacted strongly.
71	8/19	Anethol, 10 ml. dispersed with Tergitol.	P	T	-	Curiosity to smell in first period--licorice-like.
72	8/20	Skipjack flesh: 250 ml. stock extract.	P	T	XXX	
73	8/20	Skipjack flesh: 500 ml. stock extract, heated at 85° C for 1/2 hour.	P	T	XX	
74	8/20	Whole beef blood, 250 ml.	P	T	-	Repellent effect.

Expt. No.	Date 1952	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
75	8/20	Skipjack flesh, 500 ml. stock extract, heated at 69° C. for 1/2 hour, centrifuged: washed residue.	P	T	X	No great excitment.
76	8/20	Same as No. 75: centrifugate.	P	T	XX	Feeding reaction.
77	8/20	Skipjack flesh, 500 ml. stock extract, boiled 1/2 hour: filtrate.	P	T	XX	Feeding reaction, yellowfin participated.
78	8/21	Skipjack flesh, 1000 ml. stock extract, boiled 1/2 hour, centrifugate.	P	T	XX	
79	8/21	Same as No. 78: residue.	P	T	X	Very weak but positive reaction.
80	8/21	Skipjack flesh, 250 ml. stock extract.	P	T	X	Weak but positive.
81	8/21	Skipjack flesh, 250 ml. stock extract, old.	T	T	XXX	Fish in tank responsive after their rest.
82	8/21	Skipjack flesh, 250 ml. stock extract, new.	T	T	XX	"
83	8/22	Skipjack flesh, 600 ml. stock extract, boiled 15 min., centrifugate saturated with NaCl stood overnight, centrifuged: centrifugate.	P	T	XXX	Delayed reaction.

Expt. No.	Date 1952	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
84	8/22	Skipjack flesh, 600 ml. stock extract, boiled 15 min., centrifugate boiled one hour: centrifugate of second boiling.	P	T	XXX	Good feeding reaction.
85	8/22	Skipjack flesh, 600 ml. stock extract, boiled 15 minutes: centrifugate.	P	T	XX	Only 5 of 11 fish participating.
86	8/22	Part of No. 83: 100 ml. boiled, salted centrifugate.	T	T	XXX	Verifies No. 83.
87	8/22	Skipjack flesh, 100 ml. stock extract (putrefied).	T	T	X	Very slight reaction.
88	8/25	Same as No. 83, centrifuge residue.	P	T	-	
89	8/25	Animal Protein Factor (equivalent in growth effect to vitamin B_{12}), 50 g.	P	T	-	
90	8/25	Skipjack flesh, 1000 ml. stock extract, dialyzed 9/21 with 2 liters dist. water, changed once: dialyzate.	P	T	X	Slight reaction: feeding reaction.
91	8/25	Same as No. 90: residue after dialysis.	P	T	XX	Better reaction, fish fed heavily previous day and not too responsive; yellowfin also reacting.
92	8/25	Animal Protein Factor, 25 g.	T	T	-	Visual attraction only.

Expt. No.	Date 1952	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
93	8/25	Skipjack flesh, 300 ml. stock extract (putrefied).	T	T	X	Reaction apparent but weak.
94	8/26	Ulua blood, 65 ml. plus 5 cc. 4% citric acid.	P	T	X	Positive though weak.
95	8/26	Skipjack blood, 75 ml. plus 5 cc. 4% citric acid	P	T	XX	Stronger; feeding reaction.
96	8/26	Ulua flesh, 100 g. extracted with 300 ml. dist. water: centrifugate.	P	T	X	Positive but weak, lasted only short time.
97	8/26	Skipjack flesh, 250 ml. stock extract.	P	T	XX	Yellowfin also reacted, feeding reaction.
98	8/26	Barracuda flesh, 100 g. extract with 300 ml. dist. water: centrifugate.	P	T	(X)	Very slight reaction noted.
99	8/26	Aholehole whole, 250 g. extracted with 500 ml. dist. water, supernatant liquid boiled: centrifugate.	P	T	XXX	Great excitement, yellowfin reacting.
100	8/26	Same as No. 99: residue from centrifuging upper part, mostly skin, etc.	P	T	XXX	Great excitement in 2nd and 3rd periods.
101	8/28	dl-Serine, 3 g.	P	T	?	Fish showed curiosity, but no speeding up or noticeable attraction.

Expt. No.	Date 1952	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
102	8/28	l-Asparagine, 3g.	P	T	-	As above, sensed but no noticeable attraction. Fish erratic.
103	8/28	Arginine, 3 g.	P	T	-	As above.
104	8/28	dl-Serine, 1 g.	T	T	-	Absolutely no attraction.
105	8/28	Aholehole, whole 250 g. extracted with 500 ml. dist. water, not boiled: centrifugate.	T	T	X	Slight attraction.
106	8/28	Skipjack flesh, 250 ml. stock extract.	T	T	XX	
107	8/28	Skipjack flesh, 100 ml. of boiled, salted, stock extract, concentrated to about 1/3 original volume by boiling.	T	T	XXX	
108	8/29	dl-Serine, 3 g.	P	T	-	No reaction whatsoever.
109	8/29	Creatine, 3 g.	P	T	-	" " "
110	8/29	Nucleic acid, 3 g.	P	T	-	" " "
111	8/29	Skipjack flesh, 250 ml. stock extract.	P	T	X	Reaction positive but not strong.
112	8/29	Skipjack flesh, 3000 ml. stock extract, siphoned in over period of 48 min. to set up gradient.	P	T	XXXX	Strong and persistent reaction and attraction, fish running up gradient; time in first 1/10 of tank.

Expt. No.	Date 1952	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
113	9/2	Skipjack flesh, 2 liters stock extract; boiled; centrifuged; centrifugate treated with NaCl; centrifuged; centrifugate boiled to residue (plus NaCl); dissolved in sea water.	P	N	XX	Obvious reaction with feeding; tunny fighting with yellowfin.
114	9/2	Skipjack flesh, 1 liter stock extract; residue of dialysis boiled to gummy residue: portion soluble in 95% alcohol.	P	N	X	Strong in first period only.
115	9/2	Same as No. 114: portion insoluble in 95% alcohol.	P	N	(X)	Slight reaction in upper tenth of pond?
116	9/2	Same as No. 114: white crystals insoluble in 95% alcohol.	P	N	X	Definite reaction with feeding.
117	9/2	Skipjack flesh, 250 ml. stock extract.	P	N	XXX	Most vigorous reaction of the day.
118	9/4	Skipjack flesh, stock extract, 1000 ml. dialyzate boiled down to 10 ml.	P	vW	XXX	Yellowfin also reacted strongly.
119	9/4	Tuna fish meal, 10 g. boiled with 250 ml. water for 10 min.: filtrate.	P	vW	XX	
120	9/4	Tuna fish meal, 10 g. suspended in 250 ml. water, not boiled.	P	vW	XX	Perhaps not as strong a reaction as in No. 119.
121	9/4	Skipjack flesh, 2000 ml. stock extract, centrifugate salted, boiled to 10 g. residue, redissolved in water.	P	vW	X	Tide high, water murky, observation difficult.

Expt. No.	Date 1952	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
122	9/4	Skipjack flesh, 250 ml. stock extract: filtrate.	P	vW	XX	Tide high, water murky, observation difficult.
123	9/9	Skipjack flesh, 1000 ml. stock extract, dialyzate boiled to dryness, residue extracted with absolute alcohol.	P	vW	XX	Yellowfin also reacted.
124	9/9	Same as No. 123, alcohol insoluble portion.	P	vW	(X)	Doubtful; no reaction from yellowfin.
125	9/9	Skipjack flesh, 1000 ml. stock extract, boiled; residue (watery solution) purified by boiling with activated carbon, 1 g. gummy residue redissolved.	P	vW	X	
126	9/9	Skipjack viscera, 150 ml. juices, preserved with 2% SO_2 (H. T. P.)	P	vW	XXXX	Very strong reaction by both species.
127	9/9	Glycyl-glycine, 1 g.	P	vW	-	
128	9/9	Skipjack flesh, 250 ml. stock extract: filtrate.	P	vW	(X)	High tide; observation difficult.
129	9/11	Lecithin (animal), 2.5 g.	P	vW	-	
130	9/11	Skipjack flesh, 1000 ml. stock extract, evaporated, boiled with 5 g. activitated carbon, filtered, treated with 5 g. activated Al, evaporated to dryness; dark gum redissolved.	P	vW	X	

Expt. No.	Date 1952	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
131	9/11	Ambergris, 2.5 g.	P	vW	-	
132	9/11	Pregnenolone, 1 g.	P	vW	-	
133	9/11	Skipjack flesh 1000 ml. stock extract, evaporated, boiled with 5 g. activated carbon, filtered, treated with 5 g. anion exchange resin, evaporated to light gum, redissolved.	P	vW	XXX	Strong reaction in beginning, yellowfin also participated.
134	9/11	Skipjack flesh, 1000 ml. stock extract, evaporated, boiled with 5 g. activated carbon, evaporated to dryness, treated with 5 g. cation exchange resin, evaporated to dark gum, redissolved.	P	vW	XX	Reaction not as strong as No. 133, however, pump stopped and had to be repaired so that beginning of reaction not observed well.
135	9/11	Skipjack flesh, 250 ml. stock extract.	P	vW	XXXX	Very strong reaction, with feeding manifestation.
136	9/16	Skipjack flesh extracted with equal part water, 50 ml. filtrate, frozen, thawed.	P	vW/T	XXXX	
137	9/16	Skipjack viscera extracted with equal part water, 50 ml. filtrate, frozen, thawed.	P	vW/T	XXXX	About same as No. 136, quite strong.
138	9/16	Skipjack flesh extracted with equal part water, 50 ml., boiled, frozen, thawed.	P	vW/T	X	

Expt. No.	Date 1952	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
139	9/16	Skipjack viscera, extracted with equal part water, 50 ml. boiled, frozen, thawed.	P	vW/T	XXX	
140	9/16	Skipjack flesh extracted with equal part water, 50 ml. preserved at RT with 2% SO_2.	P	vW/T	XXX	
141	9/16	Skipjack viscera extracted with equal part water, 50 ml. preserved at RT with 2% SO_2.	P	vW/T	XX	Good in 1st period but died away quickly.
142	9/18	Skipjack flesh extracted with equal part water, 50-75 ml. boiled to dryness, extracted with petroleum ether (nothing removed), extracted with acetone (nothing removed), and alcohol; alcohol soluble part treated with absor. carbon and passed through cation column: portion not absorbed.	P	T	XXX	Feeding reaction.
143	9/18	Same as No. 142; portion absorbed on cation exchange resin and eluted with 0.15N NH_4OH.	P	T	-	
144	9/18	Skipjack viscera extracted with equal part water, 50 ml. treated with 2% H_2SO_4, kept at RT.	P	T	XX	Feeding reaction.
145	9/18	Same as No. 144, 50 ml. treated with 2% H_3PO_4, kept at RT.	P	T	X	
146	9/18	Same as above, 50 ml. frozen, thawed before using.	P	T	X	Feeding reaction.

Expt. No.	Date 1952	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
147	9/18	Skipjack flesh, 500 g. extracted with 750 ml. alcohol for 1/2 hour, 90 ml. clear, rosy-colored centrifugate.	P	T	XX	Delayed reaction.
148	9/23	Skipjack flesh, as above, extracted for 2 days with alcohol, 340 g. residue from centrifuging washed with alcohol, washed with water, extracted with 240 g. water since 9/19: centrifugate.	P	T	XXX	Feeding reaction.
149	9/23	Skipjack flesh extracted with equal parts alcohol, 100 ml. centrifugate passed through activated alumina: portion not absorbed.	P	T	XX	Feeding reaction.
150	9/23	Same as No. 149: portion absorbed on activated alumina, eluted with water.	P	T	XXX	Slightly better than No. 149; feeding reaction.
151	9/23	Skipjack flesh extracted with equal parts alcohol, 100 ml. centrifugate passed through zeolite: portion not absorbed.	P	T	XX	Feeding reaction.
152	9/23	Same as No. 151: portion absorbed on zeolite eluted with water.	P	T	XX	Slightly weaker than No. 156; feeding reaction.

Expt. No.	Date 1952	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
153	9/25	Skipjack flesh, 100 ml. alcohol extract adsorbed on activated charcoal: portion adsorbed, eluted with water, clear.	T	T	X	Reaction at first only.
154	9/25	Same as No. 153: portion not adsorbed (yellowish alcohol solution).	T	T	XXX	Feeding reaction.
155	9/25	Skipjack flesh, 400 ml. alcohol extract from No. 147; centrifuged; centrifugate ev. to dryness at R. T.; brown gum re-dissolved in 95% alcohol; passed through activated aluminum column: filtrate evap. to dryness and brown gum redissolved in water.	T	T	X	
156	9/25	Same as No. 155: eluate evap. to dryness and redissolved in water.	T	T	XXX	Most of fish did not enter attraction area until 3rd period; great feeding reaction.
157	9/25	Methionine, 0.5 g.	T	T	-	Fish exhibit curiosity?
158	9/25	Alpha Ionone, 2 ml.	T	T	-	

Expt. No.	Date 1952	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
159	9/30	Skipjack flesh, 600 g., extracted with 1000 ml. alcohol; 100 ml. treated with activated charcoal till clear, and passed through charcoal column; eluted with acetone; ca. 10 mg. small white crystals in eluate dissolved in water.	P	T	X	Weak, no feeding reaction.
160	9/30	Same as No. 159: white ppte. in alcohol residue, not adsorbed on charcoal, dissolved in water.	P	T	-	No obvious reaction.
161	9/30	Same as No. 159: portion not adsorbed on charcoal (minus white ppte. of No. 160).	P	T	XX	Feeding reaction.
162	9/30	Alcohol extract (No. 159), 100 ml., adsorbed on activated alumina: portion not adsorbed.	P	T	XX	Slightly better reaction than No. 161.
163	10/2	Skipjack flesh, 200 g. extracted once with 500 ml. alcohol (48 hr.); filtered: reddish clear filtrate.	P	T	XXXX	Great excitement, feeding reaction lasting 15 min.
164	10/2	Same as No. 163; residue extracted 2nd time with 500 ml. alcohol; filtered; residue extracted third time with 700 ml. water; filtered: clear colorless filtrate.	P	T	XX	Fish didn't enter field until end of 2nd period; then very strong feeding reaction.

Expt. No.	Date 1952	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
165	10/2	Same as No. 162: yellowish, slightly cloudy portion adsorbed on alumina and eluted.	P	T	XXX	Great excitement and feeding reaction.
166	10/7	Vitamin B_{12}, 5 mg. dissolved in water, reddish color.	P	T	-	Possibly sense material, but no noticeable reaction.
167	10/7	Skipjack flesh, 50 g., extracted with 50 ml. alcohol; adsorbed on charcoal: white crystals in eluate re-dissolved in water.	P	T	-	Fish surfaced, but no other noticeable reaction.
168	10/7	Pineapple juice, 160 ml.	P	T	-?	Fish sensed it; surfaced and turned back; no feeding.
169	10/7	Skipjack flesh, 600 g., extracted twice with 600 ml. alcohol; extracted a third time with water: 500 ml. centrifugate from water extraction.	P	T	XX	Fish didn't enter field until 2nd period; some feeding reaction but not as much as in No. 164.
170	10/9	Skipjack flesh, 100 g., extracted twice with alcohol; further extracted with alcohol for 14 hours in Soxhlet; residue extracted 12 hours with 200 ml. dist. water; centrifuged: clear colorless centrifugate.	P	T	(X)	Very slight reaction, no feeding.

Expt. No.	Date 1952	Substance and preparation	Tank or Pond	Ob-server	Reaction	Remarks
171	10/9	Alcohol extract from No. 170, adsorbed on charcoal; eluted with acetone; 20 mg. white needle-like crystals dissolved in water.	P	T	(X)	Slight sensing? No surfacing, no feeding.
172	10/9	"Alamask" (DuPont aromatic R-200.611), 2 ml. dispersed with Tergitol and emulsified in water.	P	T	-	
173	10/9	Skipjack flesh, stock solution, 250 ml. (old, deteriorated, weakened by successive freezing and removal of un-frozen solution).	P	T	X	Positive reaction in first and second period, but not thereafter.
174	10/11	"Slik", 10 ml.; (commercial fish attractant).	P	vW	-	Has cod liver oil smell. Fish noticed it but not noticeably attracted.
175	10/11	Skipjack flesh, 100 g., extracted with alcohol in Soxhlet; extracted with petrol ether; evaporated; residue dissolved in abs. alcohol and dispersed in water with tergitol.	P	vW	X	Slight attraction, no feeding reaction, speed decreased after 10 min.
176	10/11	Acetyl tryptophane, 1.5 g. dissolved in water.	P	vW	-	

Expt. No.	Date 1952	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
177	10/16	Skipjack flesh, 200 g., extracted with alcohol; evap. to dryness; fatty substances dissolved in pet. ether; pet. ether evaporated; residue taken up with abs. alcohol, dispersed in water with Tergitol: white emulsion.	P	T	X	Definite speeding up and surfacing, but rather weak reaction.
178	10/16	Skipjack flesh, 250 g., extracted with alcohol; passed through charcoal column; evap. to dryness and extracted with pet. ether; passed through Duolite C-3 Cation exchange resin: clear colorless portion not adsorbed on cation resin.	T	T	X	Slight speeding up noticed. Experiment disturbed by rain squall.
179	10/16	Same as No. 178, but passed through Duolite A-3 Anion exchange resin: clear, yellowish portion not adsorbed on anion resin.	P	T	X	Good reaction for first period, but died off quickly. Rainstorn disturbed experiment.
180	10/21	Skipjack flesh, 340 g. extracted for 9 days with 340 ml. alcohol; centrifugate.	P	T	XX	Reaction lasted for 3 periods, surfacing and feeding.
181	10/21	Same as No. 178: portion adsorbed on cation exchange resin and eluted with 5% H_2SO_4: colorless eluate.	P	T	(X)	Sensed material but very slight reaction, if any.

Expt. No.	Date 1952	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
182	10/21	Same as No. 179: portion adsorbed on anion exchange resin and eluted with 5% NaOH: yellowish eluate.	P	T	XX	Good reaction, including surfacing and feeding.
183	10/21	Skipjack flesh, 250 g. extracted with alcohol; charcoal treated; fat extracted; evap. to dryness in vacuum: portion of residue insoluble in 95% alcohol but soluble in water, purines removed as Ag salts: yellowish sol.	P	T	XX	Good reaction including surfacing and feeding.
184	10/21	Same as No. 183: portion of residue soluble in 95% alcohol; purines removed as Ag salts: yellowish solution.	P	T	X	An obvious speeding-up in first three periods.
185	10/25	Skipjack flesh, 700 g., extracted with alcohol, conc. under vacuum; fat out with pet. ether, ether removed under vacuum; passed over charcoal; adsorbed on anion exchange resin Duolite A-3; eluted with 10% NH_4OH: 1st portion of eluate.	P	vW	XX	Abudefduf also left coral and reacted.
186	10/25	Same as No. 185: 2nd portion of eluate.	P	vW	X	Tuna swam slowly, no Abudefduf reaction.
187	10/25	Same as No. 185: 3rd portion of eluate.	P	vW	-	Seemed to note substance only during 1st minute.

Expt. No.	Date 1952	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
188	10/25	Trimethylamine oxide, 1.5 g. in water.	P	vW	-	
189	10/25	Peptone "I" (Nutr. Biochem.), 1.5 g. in water.	P	vW	-	
190	10/25	l-Histidine monohydrochloride, 1.5 g. in water.	P	vW	-	
191	10/25	l-Glutamic acid, 1.5 g. in water.	P	vW	-	
192	10/28	Acetylcholine chloride, 100 mg. in water.	P	T	-	Sensed material but no surfacing or feeding reaction.
193	10/28	Skipjack flesh, 350 g., extracted with alcohol; fat out with pet. ether; through charcoal column; through anion exchange column; portion held on column and eluted with NH_4OH; evaporated in vacuum; residue redissolved in water and nitrogen compounds ppted. with phosphotungstic acid; filtrate.	P	T	XX	Good reaction with surfacing and feeding.

Expt. No.	Date 1952	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
194	10/28	Skipjack flesh, 700 g., second alcohol extraction; through charcoal column; through anion exchange column; portion eluted from column with water: whitish liquid.	P	T	XX	Fish late in entering area, but then showed good reaction, with feeding.
195	10/28	Same as No. 194: portion eluted from column with 95% alcohol.	P	T	-	No reaction at any time.
196	11/8	Skipjack flesh, extracted with alcohol; treated with lead acetate; filtered; fat removed from filtrate with pet. ether; lead removed with H_2S, and H_2S removed in vacuum; adsorbed on anion resin and eluted with 5% NH_4OH: eluate neutralized with HCl.	P	vW	-	No noticeable reaction; visibility poor.
197	11/8	Same as No. 196 to removal of H_2S: filtrate.	P	vW	XXXX	Tuna showed surfacing and feeding reaction; also Abudefduf; visibility poor.
198	11/8	Histamine diphosphate, 8 mg., free base liberated with NH_4OH.	P	vW	-	Abudefduf may have showed a slight reaction; visibility poor.

Expt. No.	Date 1952	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
199	11/18	Skipjack flesh, 80 g., extracted with water one month previously: held in reefer.	P 2/	T	XXX	Good reaction with surfacing.
200	11/18	Same as No. 196, i.e. leaded, de-leaded, fat removed, passed through anion column twice: filtrate from anion column.	P 2/	T	-	No reaction, except perhaps a slight sensing of material?
201	11/18	Same as No. 196; water eluate from No. 200 evaporated to dryness and treated with abs. alcohol: residue soluble in alcohol.	P 2/	T	XX	Fair reaction with surfacing and feeding.
202	11/18	Same as No. 201: residue insoluble in alcohol but soluble in water.	P 2/	T	-?	May be slight attraction?
203	11/18	Skipjack flesh extracted with water, same as No. 199.	P 2/	T	XXX	Good reaction.
204	11/22	Same as No. 201: weak NH_4OH eluate evaporated to dryness, treated with abs. alcohol: residue soluble in alcohol.	P 2/	vW	-	No reaction.
205	11/22	Same as No. 204: residue insoluble in alcohol but soluble in water.	P 2/	vW	-	
206	11/22	Same as No. 201: eluate at pH 3-4, neutralized.	P 2/	vW	-	

2/ Material thrown from tower; pump not working.

Expt. No.	Date 1952	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
207	11/22	Skipjack flesh, extracted with water, ca. 100 ml.	P[2/]	vW	XXXX	Good reaction.
208	12/6	Same as No. 196: first portion of water eluate from anion column, basic in nature.	P	vW	X	Swimming speed increased, no feeding reaction.
209	12/6	Same as No. 208: third portion of water eluate from anion column, still basic in nature.	P	vW	-	
210	12/6	Same as No. 208: fourth portion of water eluate from anion column, starting to become acidic.	P	vW	-	
211	12/9	Skipjack flesh, extracted with alcohol; treated with barium acetate; filtered; filtrate treated with H_2SO_4 to ppte. $BaSO_4$ and this left in suspension: filtrate.	P	T	-?	Visibility poor; no apparent reaction, although fish did not enter field until after expt. was over.
212	12/9	Same as No. 211: residue treated with NHO_3 and barium ppted. as $BaSO_4$: filtrate.	P	T	-	Visibility ok.
213	12/9	Same as No. 196, but new anion exchanger (Amberlite IRA-400) used: first water eluate.	P	T	XX	Surfacing and feeding reactions.
214	12/9	Same as No. 213: second water eluate.	P	T	-	May have been a slight sensing.

Expt. No.	Date 1952	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
215	12/9	Skipjack flesh, 1800 g. third alcohol extract; treated with tannic acid (protein out); treated with $PbAc_2$ (protein and tannic acid out); treated with H_2SO_4 (Pb out); treated with phosphotungstic acid (purines out); treated with Ba (phosphotungstic acid out); treated with SO_4 (Ba out).	P	T	X	Very weak, if any, reaction.
216	12/9	Skipjack flesh, 1800 g. first alcohol extract, 100 ml.; treated with phosphotungstic acid; filtered; phosphotungstic acid removed with barium acetate; passed through cation column (Amberlite IR-120) to remove barium: filtrate from column.	P	T	X	Very weak. Reaction about the same as No. 215.
217	12/11	Fresh tuna stickwater from H. T. P. (high oil content): 200 ml.	P	T	XXX	Reaction good; feeding and surfacing.
218	12/11	Same as No. 216, except barium chloride used: filtrate from column, neutralized.	P	T	-	Material introduced too soon by accident, but no reaction.
219	12/11	Skipjack whole ground viscera (except gonads), preserved in 2% SO_2, self-digested; prepared 9/25/52 by H. T. P.: 200 ml. brown mixture from can.	P	T	XX	Fair reaction with feeding and surfacing.
220	12/11	Skipjack whole viscera, steamed at 20 lb. press.; preserved in 3% PO_4 in bottle; prepared 5/26/52 by H. T. P.; yellowish supernatant liquid, 125 ml.	P	T	XXX	Good reaction which was renewed when pipe was flushed after expt.

Expt. No.	Date 1952	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
221	12/11	Skipjack whole ground viscera, preserved in 3% SO_2 in bottle; prepared 5/26/52 by H. T. P.; yellowish supernatant liquid, 125 ml.	P	T	XXXX	Surfacing, speeding up, best feeding reaction yet observed.
222	12/11	Same as No. 217: fresh tuna stickwater, 200 cc. mixed with fluorescein dye.	P	T	XXX	Good reaction which could be traced with dye.
223	12/13	Skipjack flesh, 1800 g., second alcohol extract, treated with phosphotungstic acid; filtered; filtrate through cation (Amberlite IR-120) column; filtrate through anion (Amberlite IRA-400) column: first water eluate.	P	vW	-	May have sensed material at beginning.
224	12/13	Same as No. 223, but carried only through phosphotungstic acid precipitation: filtrate.	P	vW	XXXX	Tunny followed material when it drifted out through gates; other fish also reacted.
225	12/13	Same as No. 216: filtrate from cation column neutralized.	P	vW	XX	Although reaction not vigorous it was positive.
226	12/16	Skipjack viscera self-digested and preserved in 2% SO_2 (same as No. 219); 200 ml. extract plus 200 ml. 95% alcohol adjusted to pH 9 with NH_4OH; filtered; centrifuged to remove crystals: centrifugate.	P	T	X	A poor reaction, no surfacing or feeding (cf. No. 219).

Expt. No.	Date 1952	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
227	12/16	Same as No. 226: crystals, residue from centrifuging, dissolved in water.	P	T	-	
228	12/16	Skipjack viscera (same as No. 219),200 ml. water added; boiled; 200 ml. water and 1100 ml. 95% alcohol added; coagulation; filtered: filtrate, pH 7.	P	T	-	No noticeable reaction?
229	12/16	Same as No. 219.	P	T	(X)	A slight reaction when fish finally entered field in 4th period.
230	12/16	Same as No. 219 (repeat of No. 229).	P	T	X	Some reaction, but no feeding.
231	12/16	Furfuryl mercaptan 10% (Du Pont Aromatics No. 2295), dispersed in water with Tergitol, 1.5 ml.	P	T	?	The material was sensed; fish surfaced, and kept returning to area. No feeding reaction noted.
232	12/27	Skipjack viscera prep. (same as No. 219), 200 ml., treated as in No. 228; filtrate adjusted to pH 2 with H_2SO_4; phosphotungstic acid added; filtered; filtrate treated with $Pb(Ac)_2$; filtered; filtrate passed through strong cation exchanger (Amberlite IR-120): unadsorbed portion.	P	vW	(X)	Slightly excited, milling around inlet for short period; very weak reaction.

Expt. No.	Date 1952	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
233	12/27	Skipjack viscera prep. (same as No. 219), 200 ml.; 400 ml. 95% alc. added; stood 1/2 hr., filtered; phosphotungstic acid added to filtrate after adjusted to pH 2 with HCl; filtered; $Pb(Ac)_2$ added to filtrate; filtered; filtrate passed through strong cation exchanger (Amberlite IR-120); adjusted pH unadsorbed portion to pH 4; passed through strong anion exchanger (Amberlite IRA-400); unadsorbed portion.	P	vW	-	No reaction.
234	12/27	Skipjack viscera prep. (same as No. 219); treated as in No. 233; adsorbed portion from anion column.	P	vW	-	No reaction.
235	12/27	Skipjack viscera prep. (same as No. 219), 400 ml.; 1000 ml. 95% alc. added; stood 1/2 hr., filtered: filtrate.	P	vW	XXX	Strong reaction with feeding.
236	12/27	Skipjack viscera prep. (same as No. 219), 400 ml.; cooked under pressure for 1/2 hr.; filtered: filtrate.	P	vW	X	Positive but weak attraction; no feeding reaction.
237	12/30	Skipjack viscera prep. (same as No. 219); treated same as No. 233: portion unadsorbed on anion column.	P	T	-	No reaction.
238	12/30	Skipjack viscera prep. (same as No. 219); treated same as No. 234: portion adsorbed on anion column.	P	T	-	No reaction.

Expt. No.	Date 1952/53	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
239	12/30	Bigeye flesh, 100 g. plus 140 ml. water extracted, filtered: filtrate.	P	T	XX	Speeding, surfacing and feeding; visibility poor.
240	12/30	Bigeye flesh, 100 g. plus 140 ml. water, cooked under pressure 1/2 hr., filtered: filtrate.	P	T	XX	About same as No. 239.
241	12/30	Furfuryl mercaptan, 1.0 ml., dispersed with Tergitol in water.	P	T	(X)	Surfacing, speeding but no feeding, attraction?
242	1/6	Adenosine, 1.0 g., dissolved in water just prior to testing.	P	T	-	No reaction.
243	1/6	Adenosine triphosphate, disodium salt, 95% 0.1 g., dissolved in water just prior to testing.	P	T	(X)	No speeding or feeding, but continuous turning back, a sensing of material?
244	1/6	Adenylic acid (muscle), 0.1 g., dissolved water (not very soluble).	P	T	-	No reaction.
245	1/6	Adenosine diphosphate, barium salt, 0.1 g., dissolved in 0.1N HCl and Ba ppted. with 6N H_2SO_4 just prior to testing.	P	T	(X)	Speeding and turning back; a sensing of material?
246	1/6	Bigeye flesh, alcohol extract, leaded and deleaded through anion exchanger; adsorbed portion eluted and evap. to dryness; re-dissolved in abs. alc. and fractionated on alcohol anion column; adsorbed portion eluted with 250 ml. water; 10 ml. fractions collected; fractions 1-6.	P	T	(X)	Turning back, but no surfacing or feeding; visibility poor.

Expt. No.	Date 1953	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
247	1/13	Same as No. 246; fractions 7-13 incl.	P	T	?	Fish came into field in 1st period but then stayed down-stream for 3 periods. Appeared to notice material when in field in 5th period.
248	1/13	Same as No. 246; fractions 14-26 incl.	P	T	-	No reaction.
249	1/13	Coumarin, 3 g. (DuPont Aromatics R. 1697), dissolved in water.	P	T	-	No reaction.
250	1/13	Methyl anthranilate, 2 ml. (DuPont Aromatics R. 1581), dispersed with Tergitol in water.	P	T	-	No reaction.
251	1/13	Phenyl acetic acid, 2 g. (DuPont Aromatics R. 7618) dissolved in water.	P	T	-	No reaction.
252	1/13	Bigeye flesh, 125 g. extracted with water; filtered; filtrate.	P	T	XX	Surfacing speeding, milling, and feeding reaction.
253	1/13	Meat flavor, 1 ml. (SA. 6321), dissolved in water.	P	T	(X)	Seemed to sense material; kept turning back, feeding reaction.
254	1/17	Adenosine triphosphate, disodium salt (same as No. 243).	P	vW	-	No reaction.

Expt. No.	Date 1953	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
255	1/17	Adenosine diphosphate, barium salt (same as No. 245).	P	vW	-	No reaction.
256	1/17	Bigeye flesh, 1800 g. extracted with 1000 ml. 95% alcohol; filtered; filtrate treated with $Pb(Ac)_2$ and Pb removed on strong cation exchanger; unadsorbed portion adjusted to pH-4 with NH_4OH and passed through strong anion exchanger; adsorbed portion eluted with water, evaporated, re-dissolved in abs. alc.; heated to boiling; charcoal added and filtered; 1/6 of filtrate (equiv. to 200 g. flesh).	P	vW	-	No reaction.
257	1/17	Bigeye flesh, 1000 g. extracted with 1000 ml. alcohol, $Pb(Ac)_2$ added; filtered filtrate put through cation exchanger; unadsorbed portion filtered to remove ppte.; filtrate through anion exchanger and eluted with water; 1/2 adsorbed portion (equiv. to 500 g. flesh).	P	vW	(X)	A little excitement at beginning but no feeding nor attraction.
258	1/20	Skipjack viscera prep. (HTP)[3/], pepsin digested, preserved in 2% $NaHSO_3$, 200 ml.	P	T	-	No reaction.
259	1/20	Bigeye flesh, 125 g., extracted with water, filtered; filtrate.	P	T	(XX)	Characteristic reaction with feeding.

3/ Hawaiian Tuna Packers, Ltd.

Expt. No.	Date 1953	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
260	1/20	Skipjack viscera prep. (HTP) pepsin digested, 60 ml. (same as No. 258)	P	T	(X)	Possibly a slight reaction?
261	1/20	Same as No. 268 and 269; remainder of unadsorbed portion from cation column put through strong anion exchanger: unadsorbed portion equivalent to 200 g. flesh.	P	T	-	No reaction.
262	1/20	Same as No. 261: adsorbed portion on anion column eluted with 200 ml. water and tested, equivalent to 200 g. flesh.	P	T	-	No reaction or very slight.
263	1/22	Skipjack viscera prep. (HTP), pepsin digested 200 ml. (same as No. 258).	P	T	-	No reaction.
264	1/22	Skipjack viscera prep. (HTP), steam cooked (1 hr/10 lb.) filtered; clear yellow filtrate preserved in 2% $NaHSO_3$: 200 ml. tested.	P	T	XX	Very strong feeding reaction.
265	1/22	Same as No. 256, except alcohol evaporated in vacuum desicator: crystals and sludge re-dissolved and tested (equiv. to 700 g. flesh).	P	T	-	No reaction, but fish erratic in behavior.
266	1/22	Bigeye flesh, 1168 g. extracted with 95% alcohol and filtered; filtrate treated with $Pb(Ac)_2$; filtered; filtrate equiv. to 200 g. flesh treated with NaCl (ppte. not removed) and tested.	P	T	XXX	An excellent reaction, with feeding.

Expt. No.	Date 1953	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
267	1/22	Same as No. 266; except filtrate equivalent to 200 g. flesh treated with NaCl, filtered, and filtrate heated to boiling for 30 min.	P	T	(X)	Possibly a weak reaction.
268	1/22	Same as No. 266; remainder of filtrate put through strong cation exchanger; unadsorbed portion equivalent to 200 g. flesh tested (pH not adjusted).	P	T	?	No reaction except perhaps in first two periods when first turned back.
269	1/22	Same as No. 268, except unadsorbed portion was adjusted to pH-5 to 7 with dil. NH_4OH, equiv. to 200 g. flesh.	P	T	X	A positive but weak reaction; no feeding.
270	1/30	Adenosine diphosphate, barium salt (same as No. 240).	P	T	-	No reaction; pump went off.
271	1/30	Adenosine triphosphate, disodium salt (same as No. 238).	P	T	-	No reaction.
272	1/30	Same as No. 269, but equivalent to 100 g. flesh, pH adjusted to 7 with NH_4OH.	P	T	X	Speeding and attraction, but no feeding.
273	1/31	Bigeye flesh, extracted with 95% alc., $Pb(Ac)_2$ added to filtrate; filtered; clear filtrate treated with NaCl and heated in water bath for 30 min., tested (equiv. to 400 g. flesh, partially extracted).	P	vW	XXX	Strong attraction but no feeding; visibility poor.

Expt. No.	Date 1953	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
274	1/31	Same as No. 273, except filtrate, after NaCl treatment put through strong cation exchanger (IR-120): unadsorbed material, equiv. to 400 g. flesh, partially extracted. (pH dropped from 7 to 3 as a result of cation treatment).	P	vW	-	No reaction; visibility poor, many ripples.
275	1/31	Same as No. 274, except unadsorbed material from cation put through a strongly basic anion exchanger (IRA-400): adsorbed material, equiv. to 400 g. flesh, eluted with 1:50 NH_4OH and tested.	P	vW	-	No reaction; visibility poor.
276	1/31	Same as No. 274, except unadsorbed material from cation put through a weakly basic anion exchanger (A-3) and eluted with 1:50 NH_4OH: adsorbed material equiv. to 400 g. flesh.	P	vW	-	No reaction, visibility poor.
277	2/5	Same as No. 272, except pH adjusted to 2 with dil. HCl; equiv. to 100 g. flesh.	P	T	-	No reaction.
278	2/5	Same as No. 274, except filtrate put through Duolite C-2 cation exchanger: unadsorbed material equiv. to 400 g. flesh (pH dropped from 7 to 3 as a result of cation treatment).	P	T	X	A weak reaction in first period.
279	2/5	Skipjack viscera prep. (HTP), same as No. 264, 200 ml.	P	T	XXX	Good reaction with feeding

Expt. No.	Date 1953	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
280	2/5	Bigeye flesh extracted (2nd time) with 95% alc., filtered; filtrate treated with $Pb(Ac)_2$; filtered; filtrate acidified with dil. HCl; filtered; filtrate, equiv. to 250 g. flesh, tested.	P	T	X	A good reaction (possibility of hangover from No. 274 in pipe).
281	2/5	Same as No. 280 except final filtrate evap. to dryness over water bath and dissolved in abs. alc.: alcohol soluble portion, equiv. to 250 g. flesh.	P	T	XX	A good reaction.
282	2/5	Same as No. 280 except final filtrate adjusted to pH 9 with dil. NH_4OH, then put through Duolite A-3 anion exchanger: adsorbed portion eluted with 1:50 NH_4OH, equiv. to 500 g. flesh.	P	T	X	A positive reaction with slight feeding.
283	2/5	Same as No. 282; unadsorbed portion from anion exchanger, equiv. to 500 g. flesh.	P	T	XX	Good reaction with feeding.
284	2/10	Skipjack viscera prep. (HTP) (same as No. 219), 100 ml. "freeze-dried" (brown powder with fishy smell); dissolved in sea water.	P	T	-	No reaction.
285	2/10	Bigeye flesh extracted (3rd time) with 95% alc. filtered; filtrate treated with $Pb(Ac)_2$; filtered; filtrate treated with HCl; filtered; filtrate adjusted to pH 7, passed through IR-120 cation exchanger: unadsorbed portion (pH 2), equiv. to 250 g. flesh, tested.	P	T	-	No reaction.

Expt. No.	Date 1953	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
286	2/10	Same as No. 285, except unadsorbed portion from cation adjusted to pH 8 with NH_4OH, equiv. to 250 g. flesh, tested.	P	T	X	A good reaction with feeding.
287	2/10	Same as No. 285: adsorbed portions from cation exchanger of No. 280 and No. 281, eluted with 1:50 acetic acid, equiv. to 500 g. flesh, tested.	P	T	XX	Stronger reaction but not much feeding.
288	2/17	Skipjack viscera prep. (same as No. 219), 200 ml., suspended in 200 ml. 95% alc., adjusted to pH 10 with NH_4OH; filtered: filtrate.	P	T	(X)	Some surfacing and perhaps sensing, but no positive reaction.
289	2/17	Skipjack viscera prep. (same as No. 219), 200 ml., suspended in 200 ml. 95% alc., adjusted to pH 2 with conc. HCl; filtered: filtrate.	P	T	(X)	Surfacing but no other good indications of positive reaction.
290	2/17	Skipjack viscera prep. (same as No. 219), 350 ml.: untreated.	P	T	XX	Violent reaction in first 2 periods, with feeding.
291	2/21	Yellowfin flesh extracted with water (HTP) Drum No. 1 (100 lb. to 50 gl.) 5 hours, preserved with 2% $NaHSO_3$, 100 ml.	P	T	XX	Good reaction with feeding; didn't come into field at first.
292	2/28	Bigeye flesh, 500 g. extracted with 95% alc. (1st time); filtered; filtrate adjusted to pH 2 with dil. HCl tested.	P	vW	XX	Visibility poor; feeding reaction; attraction apparent.

Expt. No.	Date 1953	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
293	2/28	Same as No. 292 except pH-7.	P	vW	XX(X)	Somewhat stronger reaction than No. 292.
294	2/28	Same as No. 292 except adjusted to pH-10 with dil. NH_4OH.	P	vW	XX(X)	About same reaction as No. 293.
295	2/28	Skipjack viscera prep. (same as No. 219), 200 ml. treated with 200 ml. 95% alc., adjusted to pH-2 with HCl; filtered: filtrate.	P	vW	(X)	Sensing but attraction doubtful.
296	2/28	Same as No. 295, except pH-7.	P	vW	X	Definite but weak attraction.
297	2/28	Same as No. 295, except adjusted to pH-10 with dil. NH_4OH.	P	vW	X(X)	Definite but weak attraction; stronger than No. 296.
298	3/7	Bigeye flesh, extracted with 95% alc. (2nd time); filtered; filtrate adjusted to pH-3 with dil. HCl; filtered; filtrate divided into 6 equal parts (each equiv. to 250 g. flesh); Part 1, adjusted to pH-7 with dil. NaOH.	P	vW	(X)	Sensed during 1st. period, but no feeding; remained deep.
299	3/7	Same as No. 298; Part 2, adjusted to pH-10 with dil. NaOH.	P	vW	XX	Fairly strong reaction; definite attraction and feeding.

Expt. No.	Date 1953	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
300	3/7	Same as No. 298; Part 3, adjusted to pH 10 with dil. NH_4OH.	P	vW	(X)	Excited during 1st period only.
301	3/7	Same as No. 298; Part 4, 0.2 g. $MgCl_2$ added; made basic with dil. NH_4OH; stood overnight; filtered to remove ppte.: filtrate.	P	vW	-	No reaction.
302	3/7	Same as No. 298; Part 5, adjusted to pH 1, boiled 15 min. (to release labile PO_4); neutralized with NaOH, filtered: filtrate.	P	vW	(X)	Sensing but no great excitement; definite reaction in 1st period.
303	3/7	Same as No. 298; Part 6, adjusted to pH 1, boiled 15 min., 0.2 g. $MgCl_2$ added; adjusted to pH 9 with NH_4OH, stood overnight; filtered to remove ppte.: filtrate.	P	vW	XX	Definite attraction with feeding; fish remain deep for most part.
304	3/19	Yellowfin flesh extract (HTP-Drum No. 2; 2/24/53?); 100 lb. to 50 gal. water, extracted for 5 hours, preserved with 2% $NaHSO_3$: 200 ml.	P	T	X	Good reaction for 1st period only: no feeding.
305	3/19	Yellowfin flesh extract (HTP-Drum No. 3; 3/4/53); 100 lb. to 50 gal. water, extracted for 5 hours, preserved with 2% $NaHSO_3$: 200 ml.	P	T	(X)	Slight attraction at beginning?
306	3/19	Skipjack viscera extract (HTP-Drum No. 4; 3/13/53) 100 lb. to 5 gal. water, extracted for 5 hours, preserved with 2% $NaHSO_3$: 200 ml.	P	T	(X)	Very slight, if any attraction.

Expt. No.	Date 1953	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
307	3/19	Yellowfin flesh extract; same as No. 291 (HTP-Drum No. 1; 2/18/53) 100 lb. to 5 gal. water, extracted for 5 hours, preserved with 2% $NaHSO_3$: 200 ml.	P	T	X	Good reaction during 1st period only; pump off in 4th and 5th period.
308	3/19	Repeat of No. 304 (Drum No. 2); 200 ml. (thrown into pond).	P 4/	T	XXX	Very good reaction, persisting for 1/2 hour. Surfacing, milling, speeding, feeding.
309	3/19	Repeat of No. 305 (Drum No. 3); 200 ml. (thrown in).	P 4/	T	(X)	Didn't enter field until 3rd period, weak, if any, reaction.
310	3/19	Second repeat of No. 305 (Drum No. 3); 200 ml. (thrown in).	P 4/	T	X	Weak reaction.
311	3/19	Repeat of No. 306 (Drum No. 4); 200 ml. (thrown in).	P 4/	T	X	Weak reaction.
312	3/19	Repeat of No. 307, same as No. 291 (Drum No. 1); 200 ml. (thrown in).	P 4/	T	XX	Good reaction, once material sensed, with feeding.
313	4/7	Skipjack flesh, 378 g. extracted with 300 ml. water and 83 ml. alcohol; filtered; filtrate treated with $Pb(Ac)_2$; filtered; activity of filtrate adjusted to pH-2; a.m.	P 4/	T	XXXX	Violent reaction with feeding activity and fighting with Caranx.

4/ Material thrown in from tower; noise of pump distracts fish.

Expt. No.	Date 1953	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
314	4/7	Same as No. 313, pH 7; a.m.	P[4/]	T	XX	Material drifted rapidly to gates; sensed by Abudefduf as well as tunny.
315	4/7	Same as No. 313, pH 10; a.m.	P[4/]	T	XX	Delayed reaction with feeding activity. Caranx also reacted.
316	4/7	Same as No. 313, pH 2; p.m.	P[4/]	T	XX	Caranx also reacted; tunny dashing up and down pond.
317	4/7	Same as No. 313, pH 7; p.m.	P[4/]	T	XX	Feeding activity.
318	4/7	Same as No. 313, pH 10; p.m.	P[4/]	T	X	Weak reaction, but with some feeding activity.
319	4/11	Same as No. 313, pH 2, a.m.	P	vW	XX(X)	
320	4/11	Same as No. 313, pH 7; a.m.	P	vW	X	
321	4/11	Same as No. 313, pH 10; a.m.	P	vW	(X)	
322	4/11	Same as No. 313, pH 2; p.m.	P	vW	X(X)	
323	4/11	Same as No. 313, pH 7; p.m.	P	vW	-	Material lost through faulty hose connection at funnel.

Expt. No.	Date 1953	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
324	4/11	Same as No. 313, pH-10; p.m.	P	vW	(X)	Three-fourths of material lost as in No. 323.
325	4/14	Same as No. 313, pH-10; a.m.	P	T	X(X)	Little surfacing, no feeding; visibility poor, Caranx reacting.
326	4/14	Same as No. 313, pH-2; a.m.	P	T	X	Remained deep. Visibility poor.
327	4/14	Same as No. 313, pH-2; a.m.	P	T	X	"
328	4/14	Condensed herring solubles (B.C.P.)[5/], 200 ml.	P	T	O	No reaction, but fish didn't enter area after first period.
329	4/14	Same as No. 328	P	T	O	No reaction.
330	4/14	Same as No. 313, pH-10; p.m.	P	T	X	Didn't enter area until 4th period.
331	4/14	Same as No. 313, pH-2; p.m.	P	T	(X)	Sensed material but only turned back once and circled.
332	4/14	Same as No. 313, pH-7; p.m.	P	T	(X)	Slight reaction with feeding; visibility poor; raining.

5/ British Columbia Packers, Ltd.

Expt. No.	Date 1953	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
333	4/18	Same as No. 313, pH 10; a.m.	P	vW	(X)	
334	4/18	Same as No. 313, pH 2; a.m.	P	vW	X	
335	4/18	Same as No. 313, pH 7; a.m.	P	vW	XX(X)	Reaction strong in first period.
336	4/18	Same as No. 313, pH 10; p.m.	P	vW	-	Fish didn't enter area during test periods.
337	4/18	Same as No. 313, pH 2; p.m.	P	vW	X	
338	4/18	Same as No. 313, pH 7; p.m.	P	vW	X(X)	
339	4/21	Same as No. 313, pH 7; a.m.	P	T	XX	Slight feeding activity, Caranx also reacting.
340	4/21	Same as No. 313, pH 7; plus furfuryl mercaptan; p.m.	P	T	XX	Tunny didn't enter area until 4th period; Caranx reacting strongly.
341	4/21	Same as No. 313, pH 10; p.m.	P	T	(X)	Didn't enter area until 3rd period; speeding only.
342	4/21	Same as No. 313, pH 2; p.m.	P	T	XXX	Strong reaction with feeding activity.
343	4/25	Same as No. 313, pH 7; a.m.	P	vW	XXX(X)	

Expt. No.	Date 1953	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
344	4/25	Same as No. 313, pH 10; a.m.	P	vW	X(X)	
345	4/25	Same as No. 313, pH 2; a.m.	P	vW	(X)	
346	4/25	Same as No. 313, pH 7; p.m.	P	vW	XXXX	
347	4/25	Same as No. 313, pH 10; p.m.	P	vW	XX(X)	
348	4/25	Same as No. 313, pH 2; p.m.	P	vW	XX(X)	
349	4/28	"Fish lure" (B.C.P), 200 ml.	P	T	X	Reaction during 1st period only.
350	5/19	Skipjack viscera extract (HTP-Drum No. 5; 5/11/53), 300 ml. a.m.	P	T	X	Didn't enter until 3rd period, Caranx reacting.
351	5/19	Same as No. 350; a.m.	P	T	(X)	Caranx reacting better than tunny.
352	5/19	Frozen ground skipjack viscera (HTP-5/11/53), 1 lb. extracted for 1 hour in water, a.m.	P	T	X	Visibility poor.
353	5/19	Same as No. 352, p.m.	P 6/	T	XX	Good feeding activity.
354	5/19	Same as No. 305 (HTP-Drum No. 3 3/4/53) (drawn after sea test of 5/13/53), 500 ml.; p.m.	P 6/	T	X	Slight feeding activity.

6/ Material thrown from tower, power off.

Expt. No.	Date 1953	Substance and preparation	Tank or Pond	Observer	Reaction	Remarks
355	5/19	Same as No. 306 (HTP-Drum No. 4; 3/13/53) (drawn after sea test of 5/13/53); 500 ml.; p.m.	P 6/	T	(X)	No excitement, sensing only.
356	5/19	Same as No. 352.	P 6/	T	XX	Good reaction from both tunny and Caranx.